DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01424-27 OCD

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Behavioral Modulation by the Limbic System in Man

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: :	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	R. Davidson, Ph.D.	IRTA Fellow	CNU, OCD, NINDS
	A. August, M.A.	Psychologist	CNU,OCD, NINDS
	C. Kufta, M.D	Medical Officer	SNB, NINDS
	S. Sato, M.D.	Medical Officer	EEG, OCD, NINDS
	W. Theodore, M.D.	Medical Officer	CES, ERB, NINDS

COOPERATING UNITS *(if any)*

Surgical Neurology Branch, DIR, NINDS
Epilepsy Research Branch, DIR, NINDS

LAB/BRANCH

Office of the Clinical Director

SECTION

Clinical Neuropsychology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Personality and mood characteristics were studied in epileptic patients before and after unilateral left or right temporal lobectomy. The research examined the role of the limbic system in emotional perception and expression, and how brain injury alters these functions.

Left temporal lobectomy (LTL) patients presented an avoidant profile with features of anxiety and dysthymia. Right temporal lobectomy (RTL) patients were overtly expressive, with histrionic features. This pattern is consistent with the hyper-hypoarousal model which accounts for different emotional reactions following left and right brain injury. This also confirms observations made following intracarotid amytal injection where euphoria follows right, and dysphoria, left injection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 01245-28 OCD

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
EEG Learning Correlates Using Scalp and Intracranial Electrodes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio, Ph.D	Unit Chief	CNU, OCD NINDS
Otners:	S. Sato, M.D	Medical Officer	EEG, OCD, NINDS
	A. August, M.A.	Psychologist	CNU, OCD, NINDS
	C. Kufta, M.D.	Medical Officer	5NB, NINDS

COOPERATING UNITS *(if any)*
Surgical Neurology Branch, DIR, NINDS

LAB/BRANCH
Office of the Clinical Director

SECTION
Clinical Neuropsychology Unit

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Personality and mood characteristics were studied in epileptic patients before and after unilateral left or right temporal lobe resection or intracarotid amytal injection. Physiologic reactions (skin conductance and EEG) were monitored during evocative procedures. The research examined the role of the left and right temporal lobes in emotional perception and expression, and how brain injury alters these functions.

Right temporal lobectomy (RTL) patients showed a pattern of hypoarousal with rapid habituation whereas left temporal lobectomy (LTL) patients showed hyperarousal and increased vigilance. In a behavioral paradigm, RTL patients were more responsive to failure than LTL patients who showed dulled activation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 00200-39 OCD

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cognitive and Emotional Profile of Neuropsychiatric Disorder

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P. Fedio, Ph.D.	Unit Chief	CNU, OCD, NINDS
Others:	A. August, M A.	Psychologist	CNU, OCD, NINDS
	R. Davidson, Ph.D.	Psychologist	CNU, OCD, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Office of the Clinical Director

SECTION
Clinical Neuropsychology Unit

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiments using brain stimulation, PET imaging and behavioral procedures were initiated to identify the neuroanatomic basis underlying different types of memory and language disorders exhibited by patients with neurologic disorders.

Patterns of dissociation were elicited during brain stimulation from lateral cortical and thalamic sites. Recall for both the anomia and target name differed suggesting that the temporoparietal cortex is critical for semantic and episodic memory With basolateral stimulation, the patient recalled both the anomic episode and misnamed target object. Activation of semantic systems is critical to ensure later recall.

A unique group of patients with language mediated by the basolateral temporal cortex was identified and showed anomia, clustering defects, and impoverished vocabulary. The group may be at risk for post-operative dysphasia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS02151-19 NSS

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Memory Storage in Neural Networks

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.L. Alkon Medical Officer BNP, DIR, NINDS

Others: NINDS: T Nelson, Chemist; D Lester, Vis Assoc; C Collin, Vis Assoc; R Etcheberrigaray, Vis Assoc; L Wang, Vis Assoc; G Adam, Vis Assoc; S Moshiach, Vis Assoc; B Schreurs, Senior Staff Fellow; J Olds, Senior Staff Fellow; E Ito, Vis Fellow; CJ Lee, Vis Fellow; Y-F Han, Vis Fellow; D McPhie, IRTA Fellow; J Schachter, Staff Fellow; KL Blackwell, Guest Res; M Boakye, Gues Res; J Mancilla, Spec Volunteer; K Kusuzaki, Spec Volunteer

COOPERATING NITS (if any)

Marine Biological Laboratory, Woods Hole, MA 02543 (A Kuzirian); California Institute of Technology (C. Chen); Medical Research Council, Canada (B Bank)

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP, DIR, NINDS

SECTION

Neural Systems Section

INSTITUTE AND LOCATION

Park Building, Room 431 and Building 9, Room 1W125, NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 11.0	PROFESSIONAL: 10.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The principal objective of the program is to define molecular and biophysical mechanisms of learning and memory. Emphasis is placed on learning and memory which can be related to human cognition. Ultimate goals of such research are to arrive at clinically meaningful interventions and to design and construct artificial intelligence which has advanced learning and memory capabilities. With human cognitive function as the principal frame of reference, the research focuses on associative processes (such as Pavlovian conditioning) rather than non-associative behavioral modifications (such as sensory adaptation, habituation, arousal, and sensitization). The biological basis of learning and memory is of interest at several levels of complexity: behavior, neuronal systems, neuronal architecture and membranes and molecular transformations. To reconstruct the physiology involved (and to model it in artificial contexts) it is necessary to use both "simple system" preparations such as the nudibranch mollusc *Hermissenda crassicornis* as well as "complex system" preparations such as rabbits and rats. The molluscan work thus far has yielded the first unequivocal biological record of an associative memory. This record consists of persistent transformations of specific ionic channels. Because these records have been found within the membranes of identified single neurons it has proven possible to define biochemical pathways which regulate such long-term membrane modifications as well as to analyze how this biological memory record is expressed by the integrative functions of an entire neuronal system. The work on the vertebrate brain offers two essential opportunities. First, the generality of mechanisms determined for much less evolved species can be tested. Remarkably, the same ionic channel transformations have been shown in our program to record associative memory in the rabbit as were found in *Hermissenda*. Rabbit and now rat neural systems have also provided sufficient quantities of tissue so that conditioning-specific alterations of critical enzymatic (e.g., protein kinase C) pathways which control membrane excitability have recently been demonstrated. Furthermore, identical G protein substrates which regulate similar K^+ channels, intraaxonal transport, m-RNA turnover, and architecture of dendritic trees, undergo memory-specific modification in mollusc and mammals. Such biophysical and molecular parallels in mechanisms of memory storage suggest the possibility of general cellular principles of memory storage with significance for human physiology and pathophysiology as well.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02875-01 NS

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Human Dementias

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Lev G. Goldfarb, M.D., Ph.D.	Visiting Scientist	NS
Others:	Mark Dubnick, Ph.D.	Senior Staff Fellow	NS
	James Nagle	Biologist	NS
	Michael FitzGerald	Biologist	NS

COOPERATING UNITS (if any)

Paul Brown, M.D., Larisa Gervenakova, Ph.D., D. Carleton Gajdusek, M.D., LCNSS, NINDS

LAB BRANCH

Office of the Director, BNP, DIR

SECTION

Neurogenetics Section, BNP, DIR

INSTITUTE AND LOCATION

NINDS, Park Building, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[X] (a2) Interviews

[X] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Creutzfeldt-Jakob disease (CJD) is an inheritable and infectious disorder, and is prevalent in any population with a frequency of 1 per million; a portion of these cases are familial. We have studied 104 patients from 65 CJD-affected families. Two germ-line point mutations and an expanding 24-nucleotide repeat were identified in the PRNP gene on chromosome 20 in each of the familial CJD patients. Individuals carrying the mutated allele, but none of their siblings without a mutation, have eventually died of CJD. The Lod score for different mutations was 4.3 to 11 at a recombination fraction 0. CJD patients coming from high-incidence clusters of this disease in Czechoslovakia, Israel, and Chile all had a point mutation at codon 200 of the PRNP gene. In 10 studied cases of Gerstmann-Straussler-Sheinker syndrome (GSS), a point mutation in codon 102 of the PRNP gene was identified. Spongiform encephalopathy was transmitted to primates from 16 familial CJD patients with codon 200 mutation, 6 with codon 178 mutation, 3 with the expanding repeats, and 3 with GSS. Fatal familial insomnia (FFI) and familial CJD are distinct syndromes linked to the same 178 Asp substitution. Phenotypic expression is dependent on a "neutral" polymorphism at codon 129: the 178 Asn + 129 Met allele is responsible for FFI, whereas 178 Asn + 129 Val have been found exclusively in the CJD variant.

PrP protein is subject to a mutation-induced or spontaneous conformational change that makes it stable to protease degradation and capable of transforming virgin precursor molecules. These properties make PrP infectious and the disease transmissible. In our experiments, synthetic peptides homologous to several regions of the PrP sequence spontaneously formed amyloid fibrils with unique morphologic characteristics and polymerization tendencies. Peptides homologous to mutated regions of PrP exhibited enhanced fibrillogenic properties and, if mixed with the wild type peptide, produced even more abundant and larger fibrous aggregates which is viewed as the primary event leading to amyloid accumulation and disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02876-01 NS

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Movement Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI	Lev G Goldfarb, M D , Ph D	Visiting Scientist	NS
Others:	Mark Dubnick, Ph.D.	Senior Staff Fellow	NS
	Bjorn Olde, Ph D	Visiting Fellow	NS
	Michael FitzGerald	Biologist	NS
	James Nagle	Biologist	NS
	Astrid Lunkes, Ph.D.	Special Volunteer	NS

COOPERATING UNITS *(if any)*

Mark Hallett, M.D , Camilo Toro, M.D , MNB, NINDS; Joseph Higgins, M.D , Linda Nee, MPH, CNB, NINDS

LAB/BRANCH

Office of the Director, BNP, DIR

SECTION

Neurogenetics Section, BNP, DIR,

INSTITUTE AND LOCATION

NINDS, NIH, Park VBuilding, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	2 25	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[x] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genetic typing of the largest known Siberian kindred with autosomal dominant cerebellar ataxia has been completed Nine alleles were identified in this kindred at the D6S89 locus and 5 alleles at the D6S274 locus of chromosome 6p. The affected individuals in 5 of 7 separate pedigrees carry D6S89 allele-4, and 2 pedigrees apparently had a recombination between the previously identified SCA1 gene locus and this telomeric marker No recombination was observed between the SCA1 gene locus and the centromerically located microsatellite D6S274, the affected members of all 7 families carry the same D6S274 allele-3 The D6S274-allele-3/D6S89 allele-4 haplotype was detected in all affected individuals. These data suggest that there is significant linkage between the disorder and both markers, and that ataxia in this Siberian kindred is linked to the SCA1 gene on chromosome 6p The mutation has been identified as a CAG trinucleotide repeat expansion within the SCA1 coding region This result opens wide prospectives for clinical, preclinical and prenatal testing in the Siberian kindred, as well as in American families with ataxia

We estimated the effects of buspirone, a selective 5-HT_{1A} serotonin receptor agonist, for symptomatic treatment in 16 patients with cerebellar ataxia The patients took 60 mg/day of buspirone for 7 weeks The results were assessed by weekly clinical rating, self-assessment rating, motor performance and posturography We concluded that buspirone may be effective in symptomatic treatment of mild to moderate cerebellar ataxia and that a double-blind placebo-controlled study is now appropriate Experimental characterization of expressed serotonergic receptors using a number of pharmacological agents, including buspirone, is currently in progress

A large Virginia family with essential tremor combined with focal dystonia in some family members is under genetic study to find the chromosome location for genes involved in this disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02890-01 SMS*

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Calcium Channels in Vertebrate Nerve Presynaptic Terminals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	E.F. Stanley, Ph.D.	Staff Physiologist	SMS, NINDS
Others:	Xiao-Ping Sun, Ph.D.	Visiting Fellow	SMS, NINDS
	Wolfram Gottschalk	Pre-Irta Fellow	SMS, NINDS

COOPERATING UNITS (if any)

University of Iowa (P. Haydon, Ph.D.)

LAB/BRANCH

Basic Neuroscience Program

SECTION

Synaptic Mechanisms Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.75	2.75	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Calcium-dependent release of neurotransmitters is a crucial step in virtually all aspects of central and peripheral neuron function but this process remains poorly understood due, primarily, to the generally small size of nerve terminals. Previously, we have demonstrated that the calyx-type of presynaptic nerve terminal of the chick ciliary ganglion can be used to record single calcium channel activity at a presynaptic nerve terminal release face. We now report the following studies based on this preparation: (a) examination of transmitter release gating at the release site; (b) search for ligand-gated ion channels on the nerve terminals; and (c) investigation of the structure of the nerve terminal release face using atomic force microscopy (AFM). We have found that the release of a transmitter quantum can be gated by a single calcium channel opening and the influx of about 180 calcium ions. We have also obtained the first direct recordings of ATP and acetylcholine (ACh) receptors on the presynaptic nerve terminal. We have used the AFM to provide preliminary evidence for the localization of presynaptic calcium channels. Finally, in order to extend these approaches to the mammalian central nervous system, we are developing an isolated hippocampal mossy fiber terminal preparation suitable for patch-clamp recording

*Formerly in LB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02608-10 ICBU

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Ion Channels in Axoplasmic Organelles

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I	John R. Clay, Ph.D	Staff Physicist	ICBU, OD, BNP, NINDS
Others:	Keith Krebs, Ph.D.	Senior Staff Fellow	CNB, NINDS

COOPERATING UNITS (if any)

Marine Biology Laboratory, Woods Hole, MA (A. Kuzirian)

LAB BRANCH

SECTION

Ion Channel Biophysics Unit, Office of the Director, BNP, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.4	PROFESSIONAL: 1.3	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with axoplasmic organelles containing sodium and potassium ion channels which are transported along the axon via neurofilaments to various points where they fuse with the axonal membrane, thereby inserting ion channels which underlie excitability in the membrane. We have recently found that the organelles can be separated on the basis of size into anterograde and retrograde fractions via control-pore-size glass bead chromatography. We are currently investigating these two fractions with transmission electron microscopy and SDS polyacrylamide gel electrophoresis. We are also investigating the ion channels contained in each fraction by incorporating the organelles into artificial lipid bilayers and using the voltage-clamp technique to characterize the ion channels electrophysiologically.

NOTICE OF INTRAMURAL RESEARCH PROJECT | Z01 NS 01282-29 CNSS

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Population Isolates: Study of Child Growth, Development, Behavior & Learning, & Disease Patterns in Isolated & Primitive Groups

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Carleton Gajdusek, M.D.	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Supv Research Biologist	LCNSS
	Richard Yanagihara, M.D.	Medical Director	LCNSS

COOPERATING UNITS (if any)
Continued

LAB/BRANCH
Laboratory of Central Nervous System Studies

SECTION

INSTITUTE AND LOCATION
NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS- 12	PROFESSIONAL: 8	OTHER: 4

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects
[X] (a1) Minors
[X] (a2) Interviews
[X] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of human biology of vanishing primitive societies focus on neurologic development and learning patterns in diverse cultural experiments in the human condition found in such isolated groups. Opportunistic investigation of problems phrased by man in isolation is the basis of approach from which most of our studies evolved: kuru-CJD GSS-FF1, HIV (AIDS), HTLV-I slow virus infections of the CNS, aging and Alzheimer's, VE, ALS/PD. mental disease, toxic neuropathies. Techniques of molecular genetics, biochemistry, immunology, virology, and field epidemiologic, clinical, linguistic and behavioral studies in cultural isolates and genetic and/or geographically isolated primitive bands yield more easily interpretable data than in cosmopolitan societies. Data and specimens from expeditions to Micronesia, Melanesia, Polynesia, South America, Asia and Africa proved valuable in recent HIV (AIDS), HTLV-I, Hantavirus, JC virus of PML and herpesvirus, CMV and EBV studies. Studies on nutrition, reproduction, fertility, age of puberty and aging, genetic distance and pleomorphisms, unusual and odd higher cortical functions in language learning, cognitive styles, computation (calculation without words or numbers) and culturally modified sexual behavior elucidate alternative forms of neurologic functioning for man which we are unable to investigate once the natural cultural experiments in primitive human isolates are amalgamated into the cosmopolitan community of man. Foci of high incidence of kuru, ALS/PD, HTLV-I myelopathy, epilepsy, familial parkinsonism, Viliuisk encephalopathy, other CNS degenerations, hysterical disorders, schizophrenia, bipolar psychoses, neoplasms, goiter, cretinism, rheumatoid diseases, diabetes, asthma, chronic lung disease, malaria, filariasis, leprosy, cysticercosis, and other infections in these isolated groups have yielded widely significant discoveries. HFRS caused by hantaviruses in Asia, USSR, Europe and newly recognized hantaviruses in the U.S. are studied. Human evolution and adaptability to high altitude, wet or arid climes, variable food supply, mineral deficiencies, toxic exposures and responses to severe diseases or social/psychologic stress are studied in appropriate populations. Thus, HTLV-1 and HIV retroviruses as causes of CNS diseases in man were first found in isolated or socially segregated groups: high-incidence TSP focus in Tumaco, Colombia; drug-using mothers in Newark, New Jersey ; epidemic neuropathy in Cuba; and are often best studied in these isolated or socially segregated groups. We now have a proto-Melanesian variant of HTLV-I in New Guinea and Solomon Islands, of an archaic origin, not associated with monkeys at least for millenia

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 00969-29 CNSS

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chronic CNS Disease Studies: Slow, Latent and Temperate Virus Infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. Carleton Gajdusek, M.D	Chief	LCNSS
Others:	Clarence J. Gibbs, Jr., Ph.D.	Deputy Chief	LCNSS
	David M. Asher, M.D.	Research Medical Officer	LCNSS
	Paul Brown, M.D.	Medical Director	LCNSS
	Ralph M. Garruto, Ph.D.	Supv Research Biologist	LCNSS
	Richard Yanagihara, M.D	Medical Director	LCNSS
	(continued)		

COOPERATING UNITS *(if any)*

Continued

LAB BRANCH

Laboratory of Central Nervous System Studies

SECTION

INSTITUTE AND LOCATION

NINDS, Bethesda, Maryland 20892

TOTAL STAFF YEARS- 12	PROFESSIONAL: 8	OTHER: 4

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[x] (a1) Minors
[x] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies focus on causes and pathogenesis of chronic degenerative CNS disorders with emphasis on MS; Parkinson's , Pick's, Huntington's and Alzheimer's diseases; ALS/PD of Western Pacific; supranuclear palsy; other presenile dementias; spinocerebellar ataxias; epilepsy; chronic encephalitis with focal epilepsy; Viliuisk encephalopathy; muscular dystrophies; chronic schizophrenia; bipolar psychoses, autism; SSPE; PML; dialysis encephalopathy; goiterous cretinism; cysticercosis; and intracranial neoplasms.

We have defined the transmissible and nontransmissible dementias as brain amyloidoses caused by post-translational modification of a specific host precursor protein to amyloid fibril deposits. We now recognize the slow unconventional viruses causing kuru-CJD-scrapie as replicating polypeptides formed *de novo* from a normal host precursor protein, specified on chromosome 20 in man and 2 in mice. The molecular elucidation of the spontaneous configurational change to infectivity, basically a crystallographic problem, is now becoming our major target. Molecular genetic analysis of familial CJD already indicates several point mutations which enormously increase ($x10^6$) the probability of this spontaneous *de novo* conversion to an infectious polypeptide. Microbiology must now contend with a totally new paradigm for replicating, infectious, pathogenic agents in the transmissible brain amylodoses. Our studies focus on the elucidation of the molecular configurational events conferring the property of infectivity on a previously normal host precursor using MRI to elucidate the change in configuration which occurs as transmissibility is produced.

In normal aging, Alzheimer's disease (AD), and Down's syndrome, a different host precursor protein (specified on chromosome 21 in man, 16 in mice) is a cell-excreted inhibitor of growth factors. Post-translational degradation of this normal precursor forms the 42 -amino acid amyloid polypeptide which polymerizes to form the deposits of amyloid angiopathy, amyloid plaques and neurofibrillary tangles in aging, AD and Down's. This occurs in all individuals who reach their 90s. Genetic, toxic, and infectious factors may accelerate this aging brain amyloid deposition.

PRINCIPAL INVESTIGATORS: (continued)

Others:	Vasily Alekseev, M.D.	Guest Researcher
	Irina Vasilyevna Alekseeva, Ph.D.	Guest Researcher
	Larisa Cervenakova, M.D	Visiting Fellow
	Eduardo Dueñas-Barajas, M.D.	Visiting Fellow
	Trond Flaten, Ph.D.	Guest Researcher
	Lev G. Goldfarb, M.D	Visiting Scientist
	Ana Gligic, M.D.	Visiting Scientist
	Mark S. Godec, M.D	Senior Staff Fellow
	Maneth Gravell, Ph.D.	Research Microbiologist
	Don C. Guiroy, M.D.	Visiting Associate
	Masaya Hironishi, M.D	Visiting Fellow
	Stuart Isaacson, M.D.	Clinical Associate (SF)
	Elaine K. Jordan, D V.M	Staff Fellow
	Bruce K. Johnson, Ph.D.	Special Expert
	Kimbra Kenney, M.D.	Medical Staff Fellow
	Pawel P. Liberski, M.D	Guest Researcher
	Carlos A. Mora, M.D.	Visiting Scientist
	Vivek R. Nerurkar, Ph.D	Visiting Associate
	Ana Nieto-Nuez, M.D	Visiting Fellow
	Julius Rajcani, M.D.	Visiting Scientist
	Pamela Rodgers-Johnson, M.D	Visiting Scientist
	Jiri Safar, M.D.	Visiting Scientist
	Ki-Joon Song, M.D., Ph.D.	Visiting Fellow
	Patricia Valente, M.D	Visiting Fellow
	Chettem Venkateshan, Ph.D.	Visiting Scientist
	Ikuro Wakayama, M.D.	Visiting Fellow

Collaborating Units:

Andrew AJDUKIEWICZ, Fiji School of Medicine, Suva, Fiji
Obed ALEMAENA, Min. of Health and Med Services, Central Hospital, Honiara, Solomon Islands
Vasilii Prokopievich ALEKSEEV, VE Service, Ministry of Health, Sakha Republic, Iakutia
Michael ALPERS, Institute of Medical Research, Goroka, Papua New Guinea
Kei AMEMIYA, LVMP, BNP, NINDS, NIH, Bethesda, MD
Brian ANDREWS, LNP, NINDS, NIH, Bethesda, MD
V. George BABU, Christian Medical College Hospital, Vellore, India
Courteney BARTHOLOMEW, University of the West Indies, Trinidad
Jan BASTIAN, Menzies School of Health Research, Darwin, Australia
Roger BAWDON, Department of Obstetrics and Gynecology, University of Texas Med. Center, Dallas, TX
William BELLINI, Center for Disease Control, Atlanta, GA
Abraham BLANK, Universidad del Valle, Cali, Colombia
Luis CARTIER-ROVIROSA, Universidad de Chile, Santiago, Chile
Kwang-Ming CHEN, Guam Memorial Hospital, Agana, Guam
Susan CHENG, EM Facility, NINDS, Bethesda, MD
Cen-Ting CHIN, Beijing University Medical School, Beijing, PRC
M. CHOU, Case Western Reserve University, Cleveland, OH
W. CLARK, U S. Dept. of Agriculture, Mission Field, Mission, TX
David CORBIN, Queen Elizabeth Hospital, Bridgetown, Barbados
Olivia CRUZ, Guam Memorial Hospital, Agana, Guam
Mark DUNCAN, University of New South Wales, Kensington, Australia
Boris Afanasievich EGOROV, Minister of Health, Sakha Republic, Iakutia
Steven FEINSTONE, FDA, CBER, DVP, Bethesda, MD

Collaborating Units (continued):
teven FEINSTONE, FDA, CBER, DVP, Bethesda, MD
ilas FRANGIONE, New York University, New York, NY
udith FRADKIN, NIDDKD, DDEM, Bethesda, MD
Genoveffa FRANCHINI, LTCB, NCI, NIH, Bethesda, MD
eryl FREI, Georgia State University, Atlanta, GA
yo FUKATSU, Sapporo Medical College, Sapporo, Japan
)mitry GOLDGABER, State University of New York, Stonybrook, NY
llen GOLDSTEIN, George Washington University, Washington, DC
aap GOUDSMIT, University of Amsterdam, Amsterdam, The Netherlands
Matt HALTIA, University of Helsinki, Inst. of Pathology, Helsinki, Finland
liroo HOSHINO, Gunma University School of Medicine, Maebashi, Japan
HOURIGAN, U.S Dept. of Agriculture, Mission Field Sta , Mission, TX
hin-Ming HSIANG, Hubei Medical College, Hubei, PRC
Julianne IMPERATO-McGINLEY, Cornell University Med. College, New York, NY
arol L. JENKINS, Institute of Medical Research, Goroka, Papua New Guinea
rederick JENSEN, Immune Response Corporation, La Jolla, California
. Jacob JOHN, Christian Medical College Hospital, Vellore, India
.R JOHNSON, Georgetown University, Washington, DC
ong KANG, University of Ottawa, Ottawa, Canada
Manuel KOURI, Pedro Kouri Institute of Tropical Medcine, Havana, Cuba
enu B LAL, Centers for Disease Control, Atlanta, GA
o Wang LEE, Institute for Viral Diseases, Seoule, Korea
awel P. LIBERSKI, Medical Academy Lodz, Lodz, Poland
Manuel LIMONTA, Institute of Tropical Medicine, Havana, Cuba
ugene O MAJOR, LVMP, BNP, NINDS, NIH, Bethesda, MD
. MANTOR, St. Thomas Hospital, U.S. Virgin Islands
edro MAS, Pedro Kouri Institute of Tropical Medicine, Havana, Cuba
.C. MILLER, National Naval Medical Center, Bethesda, MD
MILLER, Arthritis and Rheumatism Branch, NIAMSD, NIH, Bethesda, MD
iroko MINAGAWA, Kyushu University School of Medicine, Fukuoka, Japan
ao MIYOSHI, Kochi Medical School, Kochi, Japan
wen St Cloud MORGAN, University of West Indies, Kingston, Jamaica
obin MUKHOPADHYAYA, LTCB, NCI, NIH, Bethesda, MD
arash NARANG, General Hospital, Newcastle-upon-Tyne, United Kingdom
urt NOLTE, University of New Mexico, Albuquerque, NM
alph PETERSON, Cornell University Medical College, New York, NY
PLOTZ, Arthritis and Rheumatism Branch, NIAMSD, NIH, Bethesda, MD
ernard POIESZ, Health Sciences Center, State Univ. of New York, Syracuse, NY
anley RAPOPORT, LN, NIA, NIH, Bethesda, MD
atrick REDIG, Raptor Center, St. Paul, MN
eter P ROLLER, DCT, NCI, Bethesda, MD
. ROMAN, Neuroepidemiology Branch, DIR, NINDS, NIH, Bethesda, MD
eorge RUBEN, Dept. of Biological Sciences, Dartmouth College, Hanover, NH
aruya SAITOU, National Institute of Genetics, Mishima, Japan
ndres SALAZAR, Walter Reed Army Medical Center, Washington, DC
orton SALE, Min. of Health and Med Services, Central Hospital, Honiara, Solomon Islands
nas SALK, Salk Institute, La Jolla, CA
aymond C. SANDERS, Institute of Medical Research, Goroka, Papua New Guinea
uane SCHLITTER, Carnegie Museum of Natural History, Pittsburgh, PA
hn L. SEVER, Children's Hospital Medical Center, Washington, DC
lio SOTELO, National Institute of Neurology and Neurosurgery, Mexico City, Mexico
ggy SWOVELAND, University of Maryland, Baltimore, MD

aroi SWYT, BEIB, NIH, Bethesda, MD
obert TRAUB, Smithsonian Institution, Washington, DC
heodore TSAI, Center for Disease Control, Ft. Collins, CO
ordon A.H. WELLS, Ministry of Agriculture, Fisheries and Food, Surrey, United Kingdom
harles WEITZ, Temple University, Philadelphia, PA
fanasii Ivanovich VLADIMIRTSEV, VE Service, Ministry of Health, Sakha Republic, Iakutia
sevolod Afanasiech VLADIMIRTSEV, VE Service, Ministry of Health, Sakha Republic, Iakutia
oshiro YASE, Division of Neurological Diseases, Wakayama Med College, Wakayama, Japan
lasayuki YASUI, Division of Neurological Diseases, Wakayama Med College, Wakayama, Japan
akashi YOSHIKI, Hokkaido University, School of Medicine, Sapporo, Japan
ladimir ZANINOVIC, Univsidad del Valle, Cali, Colombia

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02549-12 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Herpesvirus Infections and Nervous System Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	J R. Martin, M.D	Medical Officer	LENP, NINDS
Others:	P. Gressens, M.D	Visiting Fellow	LENP, NINDS
	W.J. Mitchell, D V M , Ph.D	Sr. Staff Fellow	LENP, NINDS
	D B. Henken, Ph.D	Sr. Staff Fellow	LENP, NINDS
	H. deF. Webster, M D	Chief	LENP, NINDS

COOPERATING UNITS (if any)

Anatomic Pathology, Texas Childrens Hosp (C. Langston, M.D.); Dept. of Pediatrics, Univ. of Alabama at Birmingham (E. Kern, Ph.D.)

LAB BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project examines nervous system diseases associated with human herpes virus infections. Agents include neurotropic herpes simplex virus types I and 2 (HSV-1, -2) and varicella zoster virus (VZV), as well as four human herpesviruses known or suspected to infect the nervous system (cytomegalovirus [CMV], Epstein-Barr virus [EBV], and human herpesvirus types 6 and 7 [HHV-6, -7]). Experimental models are used to examine mechanisms underlying production of neural lesions. Problems of particular interest are: the role of infection with HSV, VZV and other herpesviruses in the production of CNS and PNS disease, including (i) acute encephalitis, (ii) infections during nervous system development, (iii) chronic demyelination, and (iv) mechanisms of CNS arteritis and stroke induced by neurotropic herpesviruses

During FY 1993 an *in situ* polymerase chain reaction (ISPCR) method was developed to localize HSV-2 DNA sequences in tissue sections in acute and latent stages of infection in mice. With appropriate controls, it was shown that in acute infection, cell labeling in brain was similarly distributed to viral antigen in adjacent sections, while in latent infection, ISPCR labeled some cells not detected by any other method. In trigeminal ganglia, more neurons were labeled by ISPCR than by *in situ* hybridization during latent infection. The enhanced sensitivity of ISPCR over previous methods makes it possible to more completely define sites of HSV latency and persistence in neural tissues than previously possible.

A study to detect HSV DNA sequences in human neonatal autopsy tissues by PCR was completed. This technique is more sensitive than previous methods to detect HSV infection in the neonatal nervous system. It provides a basis for ISPCR tests to localize HSV DNA in human brains.

To develop new measures of vaccine efficacy in preventing neurotropic virus spread along neural pathways, mice were immunized with a recombinant vaccinia virus expressing control or HSV-1 glycoprotein D genes. The HSV recombinant elicits a high level of neutralizing antibody, protects from lethal HSV-1 intranasal challenge and reduces virus titers in neural tissues. While virus spreads to the CNS along olfactory, somatosensory, sympathetic and parasympathetic pathways in non-immunes, CNS spread in immunes is restricted to a few glial cells in trigeminal pathways and is associated with an intense lymphocytic response. Critical evaluation of vaccine efficacy for neurotropic viruses will require inclusion of histological endpoints.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01995-21 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Studies of Myelin Formation, Breakdown and Regeneration

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	H.deF. Webster, M.D.	Chief	LENP, NINDS
Others:	Q.-L. Zhang, M.D.	Visiting Fellow	LENP, NINDS
	P.-X. Lin	Visiting Fellow	LNC, NINDS
	D.L. Yao, M.D.	Visiting Scientist	LENP, NINDS
	L. Hudson, Ph.D.	Unit Chief	LVMP, NINDS
	M. Brenner, M.D.	Special Expert	LMB, NINDS

COOPERATING UNITS (if any)

Laboratory of Viral and Molecular Pathogenesis, NINDS; Stroke Branch, NINDS

LAB BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.9	1.4	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to use quantitative light and electron microscopy along with *in situ* hybridization and immunocytochemistry to study cellular and molecular mechanisms of myelin formation, breakdown and regeneration. Myelinated areas are those found in ongoing myelin breakdown rather than myelin regeneration. 1) In nerve lesions involving injury to myelinated fibers, return of function depends on successful early interactions of regenerating axons with Schwann cells. Last year, we showed that supernatants prepared from proximal nerve segments 24-48 hr after transection significantly increased mitosis of cultured Schwann cells and also significantly increased their production of laminin, an extracellular matrix component that promotes growth of regenerating axons that have been transected. This year, to investigate the source of this stimulatory effect, proximal nerve segments were bisected when removed for study at intervals after transection. While maintaining orientation, some distal and proximal halves were embedded so their distal ends could be sectioned and compared by electron microscopic study. Supernatants were prepared from other distal and proximal halves to compare their effects on cultured Schwann cell laminin production. Supernatants from both proximal and distal halves removed 24 hr after transection significantly increased laminin production when compared with supernatants prepared from control nerves. Elevations were higher with supernatants from distal halves. Furthermore, 24 hr after transection, electron micrographs of transversely sectioned distal ends of distal halves of proximal segments contained many axonal growth cones and regenerating neurites along with profiles representing retrograde degeneration of some large myelinated axons. There also were some macrophages. Myelinated axons, vessels and endoneurium in sections of the distal ends of proximal halves resembled those seen in control nerves. Since supernatants from both halves elevated Schwann cell laminin production significantly, we think that the substances responsible for the effect probably originate in axons. In distal halves, the sources may be components of growth cones and regenerating neurites. Supernatants from proximal halves do not contain components of growth cones, regenerating neurites, macrophages or degenerating myelinated axons. Their effects probably are due to substances being transported in axons after synthesis in neurons which are responding to nerve transection. 2) Studies of relative levels of myelin-related protein mRNAs in sections of concentric sclerosis lesions by semiquantitative computerized image analysis were completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02550-12 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Immunologic Mechanisms in Virally-Induced CNS Demyelination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	G.L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS
Others:	C. Ryschkewitsch, B.S.	Medical Technologist	LENP, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS
	G.S. Ault, Ph.D.	Staff Fellow	LENP, NINDS
	M. Ishaq, Ph.D.	Sr. Staff Fellow	LENP, NINDS
	H.G. Ressetar, Ph.D.	Guest Researcher	LENP, NINDS

COOPERATING UNITS (if any)

Lab. Mol. Oncol., Alton Ochsner Med. Fdn. (O. Prakash), Dept. Mol. Biol., Penn State U. (R.J. Frisque); Neurol. Serv., VAMC West LA (E.J. Singer, W. Tourtellotte), Dept. Pathol., U. Cinc. Med. Ctr. (R.D. Smith)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.9	PROFESSIONAL: 1.0	OTHER: 0.9

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This project concerns mechanisms of CNS demyelination in human disease and continues to focus on the human JC polyomavirus (JCV), the etiologic agent of progressive multifocal leukoencephalopathy (PML). PML is a frequently fatal demyelinating disease which complicates up to 5% of AIDS cases, and can signal onset of clinical AIDS. Work this year has emphasized the detection of the JC virus and BK virus (BKV) by polymerase chain reaction (PCR) in PML tissues, normal human brain tissues, human kidneys, urine, Kaposi's sarcoma, and infected hamster tissues. Notable advances have concerned the use of reverse transcription PCR (RT-PCR) to detect JCV RNA, evidence that rearrangement of the JCV regulatory region occurs within the host prior to or during onset of PML, identification of a mutation in BKV apparently leading to tubulo-interstitial nephritis and end-stage renal disease in an AIDS patient, and the detection of JCV in the urine and kidneys from multiple sclerosis (MS) patients (parts of this work are also reported under related projects from the Neurotoxicology Section). The methods for typing JCV coding regions using type-specific primers previously developed in this Section have been applied to PML cases from Japan and Germany originally reported as due to SV40. Instead, JCV Type 1 was found in the German case, and JCV Type 2 in the Japanese case. Typing on paraffin-embedded tissues from an additional 33 PML cases is underway. We have sought to confirm previous reports from this Section and elsewhere that JCV sequences are present in some normal human brains. We have also examined MS brain sections for JCV DNA. To date, the results from both normal and MS brains have been negative with either ordinary PCR, nested PCR, or with intron-differential RNA PCR. However, approximately 40% of urines from MS patients were found to contain JCV DNA, and 2 of 9 MS kidneys also harbored JCV DNA. Further characterization of these JCV-MS strains is under way to determine whether they differ significantly from previously characterized JCV-PML strains. A kidney from a case of tubulo-interstitial nephritis with end-stage renal disease in an AIDS patient has been shown to harbor both JCV and BKV. The BKV DNA amplified from this kidney was mutated in a region of the large T-antigen gene though to be involved in DNA binding based on previous reports from studies with SV40. This finding raises the specter that more dangerous forms of usually harmless DNA viruses may emerge in the context of human immunodeficiency virus type 1 (HIV-1) infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02803-04 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on the designated space below.)

Mechanism of Latency and Pathogenesis of Herpes Simplex Virus in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	W J. Mitchell, D.V.M., Ph.D	Senior Staff Fellow	LENP, NINDS
Others:	J.R. Martin, M.D	Medical Officer	LENP, NINDS

COOPERATING UNITS (if any)

H. Arnheiter, Visiting Scientist, LVMP, NINDS, W. Odenwald, Staff Fellow, LNC, NINDS

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.2	0.9	0.3

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to understand aspects of the pathogenesis of herpes simplex virus (HSV) infection in the nervous system including the mechanism by which this neurotropic virus is regulated within neuronal and non-neuronal cells during acute and long-term infections. A further objective is to understand the relationship between HSV infection and disease

During FY 1993, studies to develop and analyze transgenic mouse models of HSV-1 pathogenesis were continued. The primary goal was to investigate the mechanism by which HSV-1 replication is regulated by specific host and viral transcriptional regulatory proteins during initial epithelial and subsequent nervous system infections. Analysis of transgenic mice containing the HSV-1 major immediate early promoter sequence fused to the *E. coli* beta galactosidase coding sequence has shown that HSV-1 can persist in non-neuronal cells with expression of the immediate early promoter. Viral DNA in chronically infected non-neuronal cells is localized to inflammatory lesions and appears to be related to production of the inflammation. This observation may also apply to long-term infections of non-neuronal cells in the CNS and could afford a new mechanism by which HSV could produce chronic inflammation within the CNS.

In transgenic mice expressing the homeodomain protein Hox 1.3, we previously showed that the pathogenesis of acute HSV-1 infection was profoundly enhanced. Preliminary experiments in these mice have shown that reactivation from latent HSV-1 infection occurs more efficiently in transgenic mice expressing Hox 1.3 than in non-transgenic littermates. This observation is being followed up since it is important to know whether induction of transcription of HSV-1 immediate early genes is a key to reactivation of virus from latency in neurons

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02804-04 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nervous System Regeneration in a Herpesvirus Model

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) Name, title, laboratory, and institute affiliation)

Prinicpal Investigator:	D B Henken, Ph.D	Senior Staff Fellow	LENP, NINDS
Others:	J R Martin, M D	Medical Officer	LENP, NINDS

COOPERATING UNITS (if any)

M.E. Goldstein, Ph.D., Senior Staff Fellow, LNC, NINDS
R. Curtis, Ph.D., Regeneron Pharmaceuticals, Tarrytown, NY

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

In order to develop a new mammalian model of nervous system regeneration, for comparison with classical axotomy models, peripheral and central nervous system regenerative responses to herpesvirus infection are investigated in the mouse. Initial studies examine the effects of herpes simplex virus type-1 and type-2 (HSV-1, -2) infection on host sensory ganglia following peripheral inoculation. This experimental model mimics many aspects of human clinical herpesvirus infection and may provide insights into mechanisms of post-herpetic neuralgia.

During FY 1993, this project continued to define and examine biological changes that occur in host dorsal root ganglia (DRG) as the result of herpesvirus infection following footpad inoculation. The following issues were addressed: 1) Can neurochemical alterations, that have been demonstrated in classical regeneration models, be identified in this system? In a time course study of HSV-2 infection, alterations of growth associated protein (GAP-43), a marker usually identified with regeneration in neurons, was separately analyzed in DRG cell bodies and in their peripheral and central processes. 2) Can regenerating neurites be demonstrated in this model and do they contain GAP-43? Neurites have been observed incidentally in dorsal roots in another HSV model, but current studies aim to systematically examine this question at the ultrastructural level. Other questions include: 3) Is DRG neuronal death a general finding in HSV infection? and 4) Does EDTA tissue decalcification alter detection and quantitative evaluation of neural antigens?

Findings are: 1) GAP-43 is increased in DRG and dorsal roots 14 days following footpad inoculation with HSV-2. These initial results are further evidence that, following acute ganglionic HSV-2 infection, selective neurochemical alterations can be found in DRG neurons, and another indication that the molecules that are selectively induced may relate to neuronal regeneration in this model. 2) A study documenting 60% neuronal death in DRG following HSV-2 infection was completed. In a more limited study, evidence of neuronal loss following HSV-1 infection was obtained. 3) Quantitative and qualitative analysis showed no reduction in sensitivity of neural antigen detection in EDTA-decalcified tissues. These results promise to provide insight into the effects of HSV infection on the neurobiology of the host ganglia and its connections.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02807-04 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

JC Human Polyomavirus Infection and Tumor Induction in the Neonatal Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	H.G Ressetar, Ph D	Senior Staff Fellow	LENP, NINDS
Others:	G L. Stoner, Ph D	Section Chief	LENP, NINDS
	H.deF Webster, M D	Chief	LENP, NINDS

COOPERATING UNITS (If any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was terminated due to the departure of the principal investigator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02808-04 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cellular and Molecular Studies of Growth Factors during Myelin Breakdown and Regeneration in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	H. Webster, M.D	Chief	LENP, NINDS
Others:	D. Yao, M.D	Visiting Scientist	LENP, NINDS
	X. Liu, M.D	Visiting Fellow	LENP, NINDS
	L. Hudson, Ph.D	Staff Scientist	LVMP, NINDS
	C. Bondy, M.D	Staff Scientist	DEB, NICHHD
	M. Brenner, Ph.D	Staff Scientist	SB, NINDS
	N. West, Ph.D	IPA Researcher	LENP, NINDS
	G. Collins, M.D	Professor	SUNY

COOPERATING UNITS (if any)

Lab.of Viral and Molecular Pathogenesis DIR, NINDS. Developmental Endocrinology Branch, NICHHD; Stroke Branch, NINDS; Dept of Pathology, SUNY Health Science Center, NY

LAB BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
4.1	3.6	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goals of this project are to study the gene expression of growth factors, myelin-related proteins, and other glial proteins by glial cells during nervous system injury and regeneration. This year, studies on cuprizone-induced CNS demyelination in young mice were extended by studying relative levels of myelin-related protein mRNAs during demyelination and recovery. The results showed that during cuprizone administration, mRNAs for myelin basic protein (MBP), proteolipid protein (PLP), and cyclic nucleotide phosphodiesterase (CNP) decreased to a minimum 14-28 days after starting cuprizone. They rose while the drug was continued, indicating that some oligodendroglia escaped from cuprizone toxicity before the drug was stopped. After cuprizone treatment ceased, myelin-related protein mRNAs rose rapidly to levels that substantially exceeded those seen in littermate controls, a result consistent with the rapid demyelination that has been observed morphologically. We also compared relative mRNA and peptide levels of insulin-like growth factor-I (IGF-I), one of the binding proteins (IGF-BP-2), its receptor (IGF-I-R), and of glial fibrillary acidic protein (GFAP) produced by astrocytes during experimental cryogenic spinal cord injury and during experimental autoimmune encephalomyelitis (EAE). When focal lesions are produced in the dorsal columns of rat thoracic spinal cords by cryogenic injury, lesion margins are known to contain hypertrophic astrocytes and regenerating, remyelinated axons 30-60 days after injury. *In situ* hybridization studies with specific oligonucleotide and RNA probes showed that mRNA levels for GFAP, IGF-I and IGF-BP-2 increased in lesion margins 7-21 days after injury. Double immunostaining with cell-specific markers showed that the cells producing IGF-I mRNA and peptide were GFAP-positive hypertrophic astrocytes and not microglia or macrophages. The production of IGF-I has also been studied in EAE, a frequently studied model of the human demyelinating disease, multiple sclerosis. EAE was induced in Lewis rats; they developed characteristic clinical signs and were sacrificed 8-40 days post innoculation (dpi). Spinal cord sections were examined after treatment with histological stains, specific antisera, or either oligonucleotide or RNA probes. Elevated GFAP, IGF-I, IGF-BP-2, IGF-I-R mRNA and peptide levels were detected 14 dpi; they increased and peaked 26 dpi when lesions showed severe demyelination and early myelin regeneration. IGF-I were produced by astrocytes; oligodendroglia (the myelin-forming cells in the CNS) expressed IGF-BP-2 and IGF-I-R. Decreased expression of IGF-I and GFAP were observed during clinical recovery 26-40 dpi.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02827-03 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Identification, Characterization, and Etiologic Role of Human Polyomavirus in Neurological Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	M. Ishaq, Ph.D.	Senior Staff Fellow	LENP, NINDS
Others:	G. L. Stoner, Ph.D.	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS (if any)

Dept. of Molecular and Cell Biology, Penn State University (R. J. Frisque)

LAB BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.05	1.0	0.05

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

JC virus (JCV) causes a fatal demyelinating disease in AIDS patients known as progressive multifocal leukoencephalopathy (PML), and there is evidence that JCV establishes latency in the kidney in a significant part of the human population. This investigation was undertaken to study the latency of JCV in human central nervous system (CNS) and the possible role of the virus in multiple sclerosis (MS). Using oligonucleotide primers specific for the coding region genes (early and late), JCV DNA was amplified from brain tissues by the polymerase chain reaction (PCR). The results indicated that neither normal nor MS brain tissues contained detectable levels of JCV DNA. There are two possible explanations for these negative results. Either the virus is focally distributed and the sections which were tested in this study lacked the viral DNA, or the amount of viral DNA in the tissues was lower than the detection limit of our PCR assays. In addition an intron-differential RNA PCR was developed to detect large T-antigen and small t-antigen mRNAs in the presence of the genomic DNA which usually contaminates mRNA preparations. Using primers spanning introns, large T and small t mRNAs were detected in brain tissues from PML patients with and without AIDS and in JCV-induced hamster brain tumors. This method may find application in identifying active viral oncogenes in tumor tissue, and in distinguishing active from inactive JCV infections of the brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02847-01 LENP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Morphological and Molecular Studies of CGRP in the Gastric Wall

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	G. Jakab, M.D.	Visiting Scientist	LENP, NINDS
Others:	E. Mezey, M.D.,	Visiting Scientist	CNB, NINDS
	K. Pacak, M.D.	Visiting Fellow	CNB, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS (if any)

LAB BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The goal of this project is to study the distribution of the calcitonin gene-related peptide-immunoreactive (CGRP-IR) nerve fibers and cells in the intact rat gastric mucosa and around an experimental stress ulcer. The CGRP-IR nerve fibers branching pattern suggests the existence of columnar units of innervation that orient perpendicular to the lumen. These units may form the skeleton of a complex vasoregulatory system based on the axon reflex mechanism mediated by neuropeptides. We found a number of CGRP-producing cells (likely neutrophil leukocytes) located mainly in the lower region of the lamina propria. Our observations suggest that the effectivity of the gastric mucosal defense mechanism against chemical provocations can be improved by the involvement of leukocytes committed to produce a variety of mediator compounds. The distribution of leukocytes and their close relationship with peptidergic peripheral nerve fibers suggest that CGRP may play a role in the homing and chemotaxis of circulating immune cells. CGRP can be considered as a trophic factor regulating the gastric microcirculation, which is crucial for the maintenance and regeneration of the protective mucosal barrier. Our data confirm the hypothesis that the peripheral nervous system may collaborate with the mobile elements of the immune system associated with various organs and tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02849-02 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Molecular Biology of Human Polyomavirus Pathogenesis

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

Principal Investigator:	G S Ault, Ph.D	Senior Staff Fellow	LENP, NINDS
Others:	G L Stoner, Ph D	Chief, Neurotoxicology Section	LENP, NINDS

COOPERATING UNITS (*if any*)

LAB BRANCH

Laboratory of Experimental Neuropathology

SECTION

Neurotoxicology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.05	1.0	0.05

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The ubiquitous polyomavirus JC virus may be reactivated during prolonged immune suppression from a latent infection in kidney or other organs to a productive infection of oligodendrocytes known as progresive multifocal leukoencephalopathy (PML). Events leading to brain pathology are not known, but may include or result in alterations of the regulatory region. We have compared the promoter/enhancer structure of JC virus isolates from eleven PML brains. The duplications and deletions of the regulatory region were different in each patient, and usually only one sequence was found in each. The sites of strand breakage in the promoter were not random: four or five preferred sites or areas exist. Alignment of the JCV prototype Mad-1 regulatory region with the unduplicated archetypal structure defines six blocks of sequence, A through F. The preferred sites of strand breaks delineate these regions, although Mad-1 is an unusual promoter which contains a break site not observed in other isolates, and an additional site is targeted in several promoters. Region A, containing the TATA box, and the first half of region C, containing several enhancer elements, and region E, are consistently retained. Region B, the 23-bp "insertion" in the archetypal structure relative to Mad-1, was also retained in all 11 isolates. Region D, the 66-bp "insertion", was retained in isolates from 3 patients. Regions A and D were never duplicated, whereas regions C and E usually were duplicated or triplicated. Variation in the exact point of breakage within the preferred sites, alternate use of the sites result in sequences which are unique in each case. At the same time, the limited choice of break sites and the characteristic fates of the regions themselves result in three broad patterns of repeat sequences. The patterns do not correspond to the viral genotypes 1 and 2 defined by coding region base changes, and do not appear to be a stable feature of the virus. Rather, rearrangements appear to be generated in the host from a basic archetypal sequence. Kidneys from three of these patients contain JCV genomes with mainly archetypal promoter structures, and the low level of rearranged promoter sequences which were seen had identical sequence to that from the brain. Sequences of the coding regions from these kidneys were identical to those from the brain of the same patient, demonstrating that the same viral genome in different tissues can have two different rearranged forms of the promoter. Type-specific PCR primers were used to show that the kidneys did not contain a low level of a different type virus. Only three out of six kidneys from PML patients were JCV-positive, suggesting that the kidney is not the sole site of latent infection.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02882-01 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit within the space provided.)

Viruses as Vectors for Gene Transfer to the Nervous System

PRINCIPAL INVESTIGATOR (List other professional persons below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	J R. Martin, M D	Medical Officer	LENP, NINDS
Others:	S. Keir, Ph D	Visiting Fellow	LENP, NINDS
	W J Mitchell, D.V M., Ph.D.	Sr Staff Fellow	LENP, NINDS

COOPERATING UNITS (If any)

LAB/BRANCH

Laboratory of Experimental Neurooathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

To develop new treatment strategies for genetic diseases of the nervous system, this project, initiated in FY 1993, aims to use viruses as vectors to transfer genes into nervous system cells in animal models. This requires identification of conditions in which viruses can be introduced into appropriate neural cell populations so that they exhibit long-term genomic persistence and expression of the transferred gene, and cause little or no central nervous system (CNS) injury. Neurotropic herpes simplex virus and human adenovirus vectors will be compared for their ability to fulfill these conditions in animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02677-09LMB

PERIOD COVERED
October 1, 1992 through December 19, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Gene Activity in Astrocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Michael Brenner, Ph.D.	Special Expert	LMB, NINDS
Others:	Francois Besnard, Ph.D.	Visiting Associate	LMB, NINDS
	Yuan Su, M.D.	Visiting Associate	LMB, NINDS

COOPERATING UNITS (if any)
X. Liu, MD; D-L Yao, MD (LENP, NINDS, NIH); A. Messing (Sch. Vet. Med., Univ. Wis, Madison, WI); J. Schwartz, PhD (CNB, NINDS, NIH); S. J. Kim, Ph.D. (CPCP, NCI, NIH)

LAB/BRANCH
Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Astrocytes play important roles in the development and maintenance of the central nervous system (CNS). To understand and manipulate astrocyte function, this project addresses transcriptional control of the astrocyte-specific *gfa* gene that encodes the intermediate filament protein, GFAP, or glial fibrillary acidic protein.

Using transfection of astrocytoma cells with reporter gene constructs, multiple segments have been identified within the *gfa* promoter and upstream regions; the segments interact to control its expression. Site-directed mutagenesis is being used to pinpoint the critical specific sequences within these segments. This will be followed by isolation and study of the regulatory proteins acting at these sites.

The activity of reporter constructs is also being studied in transgenic mice. A 2,000 base pair 5'-flanking fragment of the *gfa* gene was sufficient to drive expression of a β-galactosidase reporter gene in astrocytes throughout the CNS. Deleting from this construct a *gfa* segment found unimportant for expression in cultured cells also produces expression exclusively in astrocytes, but activity is largely restricted to the cortex. These results indicate that astrocytes are heterogeneous in gene expression and that different regulatory regions of the *gfa* gene are utilized by different types of astrocytes. Projects are also underway to use the *gfa* regulatory sequence to express other genes of interest in astrocytes to study brain development and function and to produce models for human diseases. Genes currently being expressed include those encoding the amyloid precursor protein associated with Alzheimer's disease, somatostatin, TGF-β1, and a putative dominant negative GFAP.

This project was transferred to the Stroke Branch, NINDS on December 19, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02800-05LMB

PERIOD COVERED

October 1, 1992 through April 2, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Neurotransmitter Receptor Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Jurgen Wess, Ph.D	Visiting Associate	LMB, NINDS
Others:	Roberto Maggio, Ph.D	Visiting Associate	LMB, NINDS
	Zvi Vogel, Ph D	Visiting Scientist	LMB, NINDS
	Zipora Pittel, Ph D	Visiting Fellow	LMB, NINDS
	Klaus Bluml	Special Volunteer	LMB, NINDS

COOPERATING UNITS (if any)

S. Gutkind, Ph.D. (LCDO, NIDR)
C. Felder, Ph.D (LCB, NIMH)

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.4	2.0	0.4

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To elucidate the molecular basis underlying the function of G protein-coupled receptors, muscarinic acetylcholine receptors (m1-m5) were used as a model system. The molecular mechanisms involved in ligand binding, receptor activation, G protein coupling, and receptor assembly were studied by using a variety of different mutagenesis techniques. Pharmacological studies with chimeric m2/m5 muscarinic receptors showed that the three-dimensional structure of muscarinic receptors (and most likely, other G protein-coupled receptors) resembles that of bacteriorhodopsin in that the 7 transmembrane domains (TM I-VII) are arranged in a ring-like fashion such that TM I lies directly adjacent to TM VII.

Mutational analysis of the m3 muscarinic receptor led to identification of several conserved Thr and Tyr residues (location: TM III, V, VI, or VII) which are critically involved in ACh binding and agonist-induced receptor activation. Systematic mutational modification of the N-terminal domain of the third cytoplasmic loop of the m3 receptor showed that a single amino acid (Tyr254; rat m3 receptor sequence), which is found in many other G protein-coupled receptors, is essential for the efficient activation of G proteins mediating stimulation of phosphatidylinositol hydrolysis. Experiments designed to study the functional roles of amino acids that are highly conserved among all G protein-coupled receptors showed that 4 conserved Pro residues (location: TM IV, V, VI, and VII) play key roles in receptor expression, ligand binding and receptor function.

Coexpression studies with fragmented m2 and m3 receptors indicated that muscarinic receptors behave in a fashion analogous to two-subunit receptors (one subunit containing TM I-V, and the other one, TM VI and VII). In addition, coexpression experiments with mutant m3 and chimeric adrenergic/muscarinic receptors showed that muscarinic receptors can interact with each other functionally at a molecular level.

The studies described above, together with biophysical and molecular modeling studies, should eventually lead to a detailed structural model of the ligand-receptor-G protein complex which should provide a rational basis for the development of novel muscarinic drugs.

This project terminated April 2, 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02825-03LMB

PERIOD COVERED

October 1, 1992 through December 31, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Transcriptional Regulation of Enzymes Involved in Excitatory Amino Acid Neurotransmission

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	John Forbes Mill, Ph D	Senior Staff Fellow	LMB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION

Developmental Biology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to study the structure, function, and regulation of the genes for the astrocytic enzyme glutamine synthetase (GS), and the neuronal enzyme phosphate-activated glutaminase (GA) Fusion constructs have been made between the GS promoter and the chloramphenicol acetyltransferase (CAT) reporter gene. Four regions of regulatory importance have been characterized in the GS promoter: a modulator region with homology to AP2, a GRE region, a silencer region, and a second region which inhibits transcription Polymerase chain reaction (PCR)-based site-directed mutagenesis of these sites has demonstrated their importance in GS regulation, and the interactions between them. The modulator site is required for activity of both the GRE and silencer sites. The occult inhibitory region appears to act independently Double-stranded oligonucleotide probes for each of these sites have been used in electrophoretic mobility shift assays (EMSA) Proteins from nuclear extracts of positively responding primary astrocytes and HepG2 hepatoma cells can bind all three sites, while those from a negatively responding cell line, HeLa, can only bind at the GRE site. The modulator site does not bind purified AP2 protein, although it contains partial sequence homology to it. The GRE site binds the DNA-binding fragment from the glucocorticoid receptor, and can compete for binding to an idealized GRE site.

Cloning the GA gene resulted in extension of the primary clones through the rapid amplification of cohesive ends (RACE) procedure Screening a rat hippocampal library with this probe resulted in isolation of a cDNA clone for GA which extends well into the 5' untranslated region. Reverse transcription followed by PCR (RT-PCR) has been used to determine the developmental and tissue-specific expression of the GA gene.

This project terminated December 31, 1992

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02829-03LMB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Mechanisms of Transcriptional Initiation and Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Yoshihiro Nakatani, Ph.D.	Visiting Associate	LMB, NINDS
Others:	Tetsuro Kokubo, Ph D	Visiting Fellow	LMB, NINDS
	Da-Wei Gong, Ph.D	Visiting Fellow	LMB, NINDS
	Shinya Yamashita, Ph.D.	Special Volunteer	LMB, NINDS
	Kaname Saida, Ph.D.	Special Volunteer	LMB, NINDS

COOPERATING UNITS (if any)
Robert G. Roeder, Ph.D (Rockefeller University, New York)
Masami Horikoshi, Ph D (Rockefeller University, New York)

LAB/BRANCH
Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.5	4.5	0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transcription initiation factor TFIID plays a central role in transcriptional regulation by facilitating promoter responses to various activators. Several reports indicate that TFIID is the direct target for activators and is involved in transducing signals from these activators to other components of the preinitiation complex to facilitate either the assembly or activity of the complex. Although the native TFIID complex can mediate both basal and activator (or repressor)-regulated transcription in reconstituted systems, the TATA box binding subunit of TFIID (TFIIDτ/TBP) can mediate only basal transcription. Thus, TFIID subunits other than TFIIDτ must be essential cofactors for regulated transcription. To further understand the functional properties of TFIID and the molecular mechanism of transcriptional regulation, it is important to clone and express each of its subunits and to reconstitute TFIID with the individual factors.

TFIID from *Drosophila* embryo nuclear extracts were purified with anti TFIIDτ antibody affinity chromatography and seven tightly associated polypeptides (230, 110,85,62,42,28,22 kDa) were identified as candidates for TFIID subunits. To isolate the corresponding cDNAs, partial amino acid sequences of each subunit were determined and used to design oligodeoxynucleotide probes. All TFIID subunits that have been identified have been cloned. Further studies on reconstitution of the recombinant subunits will provide crucial insight into understanding the molecular mechanisms of transcriptional regulation.

This project will terminate September 30, 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02846-02LMB

PERIOD COVERED
October 1, 1992 through February 19, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Gene Transfer and Control in Cerebellar Granule Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Ron G. King, Ph.D.	Senior Staff Fellow	LMB, NINDS

COOPERATING UNITS (if any)
Evelyn Ralston (LN, NINDS)

LAB/BRANCH
Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to examine gene regulation and expression in the central nervous system, and to optimize gene transfer and antisense technology. The ultimate goal is to achieve neuron-specific expression in cerebellar granule cultures, using highly sensitive reporter gene systems. The luciferase reporter gene system was investigated using electroporation techniques. The luciferase vector, controlled by the Rous sarcoma viral promoter, gave a total of 65,000 light units versus 1,800 for the promoterless vector. Given such high levels of sensitivity, the luciferase vector was used in the construction of neuron-specific vectors. A 386-base pair fragment of the regulatory region of the GAP-43 gene has been identified as controlling neuron-specific expression. This fragment was selected to control the neuron-specific expression of the luciferase gene. Two oligonucleotide primers flanking the 386 bp region were used with rat genomic DNA as template to generate the fragment by the polymerase chain reaction (PCR). Unexpectedly, three fragments were generated, a 386, 500, and 1300 bp fragment. It has been found that these fragments were specific PCR fragments and further characterization of their properties is needed. Construction has begun on an expression vector containing the beta-galactosidase gene in tandem with a multicloning expression cassette. This will allow living transfected cells expressing beta-galactosidase to be identified by fluorescent imaging techniques with simultaneous observation of the cellular effects of the inserted cDNA or antisense construct.

This project terminated February 19, 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02881-01LMB

PERIOD COVERED
April 1, 1993 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Molecular Biology of the Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R D McKay, Ph D	Acting Chief	LMB, NINDS	
Others:	A. Brown, M.D , Ph.D.	Senior Staff Fellow	R. Josephson	Pre IRTA
	C.O Brustle, M.D., Ph.D	Special Volunteer	Y. Maeda, M.D , Ph.D.	Visiting Associate
	D Collazo	Pre-IRTA	M J Marvin	Pre IRTA
	L M Delgado-Rivera, Ph D	Visiting Fellow	S Okabe, M D , Ph D	Visiting Associate
	T.E. Hayes, Ph.D.	Senior Staff Fellow	G Vaughn	Biologist
	T G Hazel, Ph.D	IRTA	C. Vicario, Ph.D.	Special Volunteer

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION
Developmental Biology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.4	4.5	1.9

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The research program in this group is in the area of developmental neurobiology. The adult mammalian brain is composed of a vast number of different neurons. In embryonic development neurons are derived from multipotential precursor cells. When these precursors stop dividing they become committed to give specific neuronal types in a very precise pattern. This commitment step, which occurs in the few hours around the last division, controls critically important features of neuron numbers and types found in the adult brain. Our work is focussed on the molecular and cellular mechanisms regulating this process.

The key methods we currently employ include: (1) the use of transgenic mice to define DNA sequences that target gene expression to neuronal precursors; (2) dissociated cell and tissue slice culture analysis of growth factors which regulate the proliferation, survival and differentiation of cells in the embryonic brain; and (3) the use of transplanted neuronal precursors to construct chimeric brains carrying genetically engineered functional neurons.

These techniques are used to analyse the molecular mechanisms controlling the development and function of the mammalian brain. The results are applicable to understanding the genetic basis of childhood tumors and neurodegenerative diseases of the central nervous system. They may also lead to powerful new therapies to reconstruct the damaged structure found in Parkinson's, Alzheimer's and Huntington's diseases.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02884-01LMB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Identification of Genes that Specify Regional Differences in the Developing *Drosophila* Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Brian Mozer, Ph.D	IRTA	LMB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Molecular Biology, BNP, DIR, NINDS

SECTION
Development Biology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

During the development of the mammalian brain, neuronal precursor fate is specified by localized positional information. Little is known about the genes that specify these regional differences. The goal of this project is to identify genes in the Drosophila brain that function early in the formation of the visual system to specify the precursors of the optic ganglia. Genes identified in the fly might then serve as probes for identifying cognates that have similar functions during mammalian brain development.

Using classical genetic and molecular genetic approaches available in the fruit fly, a number of genes have been identified whose expression patterns in the larval brain suggests they play an early role in visual system development. The current focus of this project is: (1) the analysis of an enhancer element that activates transcription in the first optic ganglia, the lamina, in response to axon ingrowth from the developing retina, and (2) genetic and molecular analysis of the mutant *Drop*, a gene involved in controlling cell proliferation in the visual system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS 01309-28LMCN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis and Function of Glycosphingolipids and Other Glycoconjugates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	P.H. Fishman, Ph.D	Chief, Membrane Biochemistry Section	LMCN, NINDS
Others:	P.A. Orlandi, Ph.D.	Research Associate	LMCN, NINDS
	P.K. Curran	Biologist	LMCN, NINDS
	H. Patel	Co-op Education Program	LMCN, NINDS

COOPERATING UNITS *(if any)*

LAB BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.9	1.3	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ganglioside G_{M1} is the cell surface receptor for cholera toxin (CT). The pentameric B subunit of CT binds to G_{M1} whereas the A subunit of CT is involved in activation of adenylylcyclase (AC). There is a distinct lag period between toxin binding and activation of adenylylcyclase. During this phase, the A subunit is reduced to the A_1 peptide which ADP-ribosylates the stimulatory G protein (G_s) of the cyclase. We recently established that CT is oriented with its A subunit facing away from the cell surface when it binds, and that the holotoxin is internalized. In addition, we found that brefeldin A, which causes disassembly of the Golgi apparatus and disruption of intracellular membrane trafficking, is a potent blocker of CT action. Although brefeldin A does not prevent the internalization of CT, it does block its conversion to the A_1 peptide. We are now in the process of delineating the pathway by which CT enters cells, the site where A_1 is generated, and how the latter gains access to G_s. As a model, we are using human intestinal Caco-2 cells, which behave in culture as differentiated enterocytes, the natural target for CT. We are employing a combination of nondenaturing gel electrophoresis and subcellular fractionation. The goal is to identify the pathway of toxin disassembly and the site(s) where disassembly occurs. We have found that the stability of CT in solution or bound to Caco membranes was pH sensitive and began to dissociate to its A and pentameric B subunits at pH 5.5, which is the pH of endosomes. In addition, small amounts of A_1 peptide were formed from membrane-bound CT in a pH-dependent manner. The presence of a membrane-associated reductase was supported by showing the activity was sensitive to N-ethylmaleimide which alkylates sulfhydral groups.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS 02366-15LMCN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Receptor-Coupled Adenylylcyclase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P.H. Fishman, Ph.D.	Chief, Membrane Biochemistry Section	LMCN, NINDS
Others:	M.D. Pak, Ph.D.	Senior Staff Fellow	LMCN, NINDS
	X.-M. Zhou, M.D., Ph.D.	Visiting Associate	LMCN, NINDS
	P.K. Curran	Biologist	LMCN, NINDS
	D.E. Kauffman	Biologist	LMCN, NINDS
	Q.T. Hoang	Biologist	LMCN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.7	2.7	2.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to identify molecular mechanisms involved in the regulation of receptor-coupled adenylylcyclase (AC). 1) Activation of protein kinase C (PKC) by phorbol esters in different types of cells is known to result in either a desensitization or a potentiation of the receptor-coupled AC. Although the underlying basis for these opposing effects is unknown, it has been suggested that they may be mediated by different forms of PKC. We now report that exposing human neurotumor SK-N-MC cells to phorbol esters resulted in both a potentiation of AC activity and a desensitization of their β_1-adrenergic receptors (β_1AR). Using several biochemical approaches, we established that the potentiation did not involve the G proteins, G_s and G_i, which regulate AC, but most likely the catalyst itself. Interestingly, SK-N-MC also express D_1 dopamine receptors which were not desensitized by phorbol ester treatment. Based on Western blotting, SK-N-MC cells expressed only one phorbol ester-sensitive PKC, PKC-α. When the cells were exposed to phorbol ester, PKC-α was rapidly translocated from cytosol to cell membrane. We propose that the type of AC may determine whether or not potentiation by PKC occurs. This may have important implications for the mechanisms by which different cell signaling systems cross-regulate each other. 2) We have been able to confirm and extend our previous evidence that human β_1AR and β_2AR are regulated differently by agonists. Stably transfected hamster cell lines were constructed which expressed either subtype at different levels. When exposed to agonist, the cells expressing either high or low levels of β_2AR exhibited a rapid, typical pattern of desensitization of agonist-stimulated AC. Both maximum stimulation (V_{max}) was reduced and dose response (K_{act}) was shifted to lower sensitivity. By contrast, agonist-treated cells expressing high levels of β_1AR displayed no reduction in V_{max}, and cells expressing low levels only a slow, modest reduction. Both cell lines, however, exhibited a shift in K_{act}. It is believed that the latter is mediated by protein kinase A via phosphorylation of the third intracellular loop of the receptors. The reduction in V_{max} is believed to be mediated by the β-adrenergic receptor kinase via phosphorylation of the C-terminus. The difference in desensitization between the two human βAR subtypes may relate to structural differences in their C-termini which are highly divergent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02784-05LMCN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of Stimulatory Guanine Nucleotide Binding Protein Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R V Rebois, Ph D	Head, Unit on Receptor Structure and Function	LMCN, NINDS
Others:	M. Toyoshige, M.D, Ph.D	Visiting Fellow	LMCN, NINDS
	N S Basi, Ph.D	IRTA Fellow	LMCN, NINDS
	D Warner, Ph D	IRTA Fellow	LMCN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Membrane Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 4.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The stimulatory G protein (G_s) mediates activation of hormone and neurotransmitter responsive adenylylcyclase (AC). The α-subunit ($G_s\alpha$) of heterotrimeric ($\alpha\beta\gamma$) G_s has a guanine nucleotide-binding site and intrinsic GTPase activity. Activation of AC occurs when an agonist-receptor complex promotes the exchange of GDP for GTP in the nucleotide binding site of $G_s\alpha$. Nucleotide exchange causes dissociation of $G_s\alpha$ from the βγ-subunit complex ($G\beta\gamma$). $G_s\alpha$ then activates AC until GTP hydrolysis occurs and $G\beta\gamma$ reassociates with $G_s\alpha$. This model is widely accepted, and G_s subunit dissociation is a critical part of the model since $G\beta\gamma$ plays an important regulatory role in the process. However, recent experimental evidence suggests that this model is incorrect. G_s from bovine brain was activated with GTPγS, a non-hydrolyzable GTP analogue. Activation was assayed by reconstitution of AC in G_s deficient S49 cyc- membranes. Subunit dissociation was assayed by immunoprecipitating $G_s\alpha$, and determining the amount of Gβ that was coprecipitated. Using these assays, it was determined that high concentrations of $MgCl_2$ caused G_s subunits to dissociate in solution, but activation of G_s by binding GTPγS at physiological concentrations of $MgCl_2$ did not. The bacterial toxin choleragen (CT) catalytically transfers the ADP-ribose moiety of NAD to $G_s\alpha$. When $G_s\alpha$ was dissociated from $G\beta\gamma$ by high concentrations of $MgCl_2$ it could not be ADP-ribosylated by CT whether or not GTPγS was bound to $G_s\alpha$. Consequently, the G_s heterotrimer but not the free $G_s\alpha$ subunit is a substrate for CT. This makes CT useful for monitoring G_s heterotrimer formation in biological membranes. When the N-terminus of $G_s\alpha$ was modified by the addition of 23 amino acids it was able to bind GTPγS. When the modified $G_s\alpha$ was used to reconstitute S49 cyc- membranes it could not be ADP-ribosylated by CT and it was unable to activate AC. Proteolytic removal of the modifying peptide restored both $G_s\alpha$'s ability to be ADP-ribosylated by CT and to activate AC. These data suggest that activated G_s heterotrimer and not the free $G_s\alpha$-subunit mediates agonist stimulation of AC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS01808-24LMCN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Glycoproteins of Myelin in Development and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN, NINDS	Carl Lauter, Chemist
Others:	Sung Hye Yim, Ph.D.	Special Expert	LMCN, NINDS	Jeffrey Hammer, Biologist
	John Prince, Ph.D.	Sr. Staff Fellow	LMCN, NINDS	
	Zbigniew Bartoszewicz, Ph.D.	Visiting Associate	LMCN, NINDS	
	Kenichi Toda, M.D.	Visiting Fellow	LMCN, NINDS	
	Naokazu Sasagasako, M.D.	Visiting Fellow	LMCN, NINDS	

COOPERATING UNITS (if any)

Dept. Neurology, Johns Hopkins University, Baltimore, Maryland
Laboratory of Biophysical Chemistry, NHLBI

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	4.3	1.2

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The myelin-associated glycoprotein (MAG) is a member of the immunoglobulin gene superfamily that is localized in the periaxonal membranes of PNS and CNS myelin sheaths where it functions in glia-axon interactions. It occurs in two developmentally regulated isoforms with differing C-terminal tails generated by alternative splicing of its mRNA. The carbohydrate in MAG consists of a mixture of oligosaccharides, many of which are sialylated and sulfated, and which are currently being isolated and characterized. During this year, it was demonstrated that the larger apparent M_r of MAG in the dysmyelinating quaking mouse is due to greater glycosylation in the mutant, especially sialylation. The expression of MAG in cultured oligodendrocytes and Schwann cells is being studied with the ultimate objectives of identifying factors that control its synthesis and probing its function in cell-cell interactions. Although cultured primary Schwann cells do not normally express MAG in the absence of neurons, some immortalized Schwann cell lines generated in our laboratory express remarkably high levels of MAG. It has now been demonstrated that the amount of MAG expressed by these lines is greater when their rate of growth is reduced by culturing in defined media or when the cells reach high density. These findings suggest that the level of MAG expression is inversely related to the rate of cell division. MAG is phosphorylated both in the Schwann cell lines and in cultured oligodendrocytes, and in both cases the phosphorylation is catalyzed at least in part by protein kinase C and calcium-activated kinases. However, phosphorylation in the two cell types differs in that it is almost exclusively on serine residues of the small MAG isoform in Schwann cells, whereas it is on both isoforms and on serine, threonine and tyrosine residues in oligodendrocytes. Addition of exogenous GM_3 ganglioside to the culture media of oligodendrocytes stimulates the formation of processes and promotes differentiation in the direction of myelination as indicated by increased synthesis of galactocerebroside, sulfatide and MAG. The treatment also stimulates the phosphorylation of MAG, although there is generally a down-regulation of phosphorylation of most proteins in the treated cells. Since cultured oligodendrocytes normally synthesize increasing amounts of GM_3 as they differentiate in culture, it seems likely that GM_3 is an essential component of their surface membrane that is needed in the preparation to myelinate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02786-0SLMCN

PERIOD COVERED

October 1, 1992 throug September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibodies to Glycoconjugates in Neurological Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN, NINDS
Others:	Robert Farrer, Ph.D.	Senior Staff Fellow	LMCN, NINDS
	Carl Lauter	Chemist	LMCN, NINDS
	Jeffrey Hammer	Biologist	LMCN, NINDS

COOPERATING UNITS (if any)

Medical Neurology Branch, NINDS; Neurobiology and Anesthesiology Branch, NIDR

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.2	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
  [ ] (a1) Minors
  [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This area of investigation began with the demonstration of monoclonal anti-MAG antibodies in patients with mixed sensory-motor polyneuropathies occurring in associated with IgM gammopathy (paraproteinemia). It was subsequently demonstrated that these anti-MAG antibodies were all directed toward carbohydrate epitopes in MAG and cross-reacted with 19 to 28 kD glycoproteins of PNS myelin and a sphingoglycolipid, sulfate-3-glucuronyl paragloboside (SGPG). This year we have established that one of the principal 19 to 28 kD glycoprotein antigens for these human anti-MAG/SGPG antibodies is PMP-22, a glycoprotein of peripheral nerve myelin that has only recently been cloned and characterized. This suggests that PMP-22, which has been implicated in the genetic abnormalities causing Charcot-Marie Tooth disease, may also be a target of the anti-MAG/SGPG antibodies causing autoimmune neuropathy associated with gammopathy. Monoclonal antibodies that are MAG/SGPG-negative in patients with gammopathy and neuropathy frequently react with ganglioside antigens in nerve. In the current year, we have completed the characterization of a monoclonal IgA reacting with the major LM1 ganglioside of peripheral nerve myelin. Although monoclonal IgM antibodies reacting with gangliosides in patients with neuropathy are common, this is the first example of a patient with IgA gammopathy in which the antigen has been identified as a ganglioside. Little is known about the molecular mechanisms by which antibodies to acidic glycolipids (SGPG or gangliosides) in patients with demyelinating neuropathy exert their pathogenic effects. In order to probe the function of acidic glycolipids in myelination and to understand mechanisms by which the human anti-glycolipid antibodies may perturb function, we are investigating gangliosides in differentiating Schwann cells. In comparison to Schwann cells cultured in the absence of neurons in serum-containing medium on polylysine, culturing them on a basement membrane substratum (Matrigel) resulted in increased synthesis of gangliosides (predominantly GM_3), but had no effect on synthesis of the galactosphingoglycolipids characteristic of myelin. This effect of basement membrane required the presence of a serum factor and was specific for ganglioside synthesis, since the synthesis of proteins, glycoproteins and phospholipids was unaffected. Although basement membrane is well known to be required for myelination, its presence in the absence of neurons signals Schwann cells to synthesize gangliosides rather than the galactosphinglipids that are enriched in myelin

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02805-04LMCN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Immunological Aspects of Myelin Abnormalities in Neuro-AIDS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Richard H. Quarles, Ph.D.	Section Chief	LMCN, NINDS
Others:	Johanna R. Moller, M.D.	Senior Staff Fellow	LMCN, NINDS
	Jeffrey Hammer	Biologist	LMCN, NINDS
	Carl Lauter	Chemist	LMCN, NINDS

COOPERATING UNITS *(if any)*

Dept. Neurology, Johns Hopkins University, Baltimore, MD; Medical Neurology Branch, NINDS

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Myelin and Brain Development Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was undertaken to elucidate biochemical and immunological aspects of myelin disorders associated with neuro-AIDS including diffuse myelin pallor (DMP) (decreased staining with Luxol fast blue), vacuolar myelopathy and multifocal demyelination in the CNS as well as demyelinating peripheral neuropathy. Postmortem subcortical white matter samples from AIDS patients with and without dementia were analyzed for quantitative and qualitative alterations of myelin proteins, including myelin-associated glycoprotein (MAG), myelin basic protein, proteolipid protein and 2',3'-cyclic nucleotide 3'-phosphodiesterase. The biochemical results were correlated with histological and immunocytochemical observations made by our collaborators at Johns Hopkins University on adjacent sections of tissue. DMP was detected histologically in about one-half of demented patients and one-fourth of the nondemented patients. However, electron microscopic, immunocytochemical and biochemical analyses of white matter indicated little or no loss of myelin proteins in areas of prominent DMP. On the other hand, substantial conversion of MAG to a proteolytic cleavage product (dMAG) was observed in some of the AIDS samples, as had previously been found in many samples from multiple sclerosis brain. Astrocytic hypertrophy was found in some of the AIDS patients both histologically and by increased levels of glial fibrillary acidic protein detected biochemically. Significant accumulation of serum proteins was detected immunocytochemically in white matter of many of the AIDS cases, especially the demented ones, and this was supported biochemically by the presence of variable levels of haptoglobin on Western blots of AIDS samples but not of control samples. Overall, the results provide little evidence for significant demyelination or myelin pathology in subcortical white matter of AIDS brain, but suggest that blood-brain barrier (BBB) perturbation may contribute to CNS pathology in AIDS and AIDS dementia. The relationship of BBB breakdown to the proteolytic MAG/dMAG conversion observed in multiple sclerosis and AIDS brain is under investigation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02848-02LMCN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Disorders of CNS Myelin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Johanna R. Moller, M.D.	Unit Head, Sr. Staff Fellow	LMCN, NINDS
Others:	Richard H. Quarles, Ph.D.	Laboratory Chief	LMCN, NINDS
	Arun Chakrabarti, Ph.D	Senior Staff Fellow	LMCN, NINDS
	Masayuki Sasaki, M.D	Visiting Fellow	LMCN, NINDS
	Carl Lauter	Chemist	LMCN, NINDS
	Jeffrey Hammer	Biologist	LMCN, NINDS

COOPERATING UNITS (if any)

Developmental & Metabolic Neurology Branch, NINDS; Laboratory of Experimental Neuropathology, NINDS; School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology

SECTION

Demyelinating Disorders Unit, Section on Myelin and Brain Development

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.7	0.3

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Myelin basic protein (MBP) and proteolipid protein (PLP) are major proteins of compact CNS myelin, whereas myelin-associated glycoprotein (MAG) and 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) are mainly localized in associated oligodendroglial membranes. These 4 myelin proteins are differently affected in various dysmyelinating, demyelinating and remyelinating circumstances, and information about changes in these proteins can increase our understanding of the specific molecular processes going on in each individual disease. In most hypomyelinating mutant animals, MBP and PLP are decreased more than MAG and CNP, due to a greater deficiency of compact myelin then of associated oligodendroglial membranes. This is true regardless of the primary cause of the hypomyelination, e.g., a PLP gene defect (Shaking pup), a cholesterol storage disorder (CSD mice), a congenital virus infection (Border disease in sheep) or in human patients with Niemann-Pick C disease. However, the TAIEP rat expressed decreased amounts of MAG compared to other myelin proteins, and the MAG in the younger animals had a higher MW when compared to age-matched controls, most likely due to an extended presence of the immature large isoform of MAG. In caprine β-mannosidosis, MAG, CNP and PLP levels were equally decreased, but MBP was relatively spared. This might be due to the presence of large storage vesicles, which interfere with the protein transport of MAG, PLP and CNP, while MBP translation is at the site of insertion into the myelin. Biochemical and histological analysis of white matter biopsies from 2 young girls with a progressive leukodystrophy due to unknown causes, revealed all the characteristic myelin proteins and lipids, but at significantly decreased amounts. In multiple sclerosis (MS) there is preferential loss of MAG at the edges of the plaques. Much of the MAG remaining in MS tissue is in the form of dMAG, a proteolytic cleavage product formed by a myelin-associated, calcium activated neutral protease (calpain). The MAG loss in MS may be related to this protease. dMAG was also present in some patients with AIDS (see Z01 NS 02805-04 LMCN). The MAG/dMAG conversion rate in incubated myelin, purified from different species, was greatest in human myelin, rapid in myelin from other primates, and substantially slower in myelin from lower mammals such as rodents. This suggests that dMAG formation may be relevant to human demyelinating diseases. It seems that the MAG/dMAG conversion rate in purified myelin is very sensitive to the Ca^{2+} concentration in the samples and the levels of some gangliosides. Purified human calpain incubated with purified human MAG degraded the MAG totally.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS02864-02LMCN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dopaminergic, Neurotrophic Factors and Reinnervation of the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	John W. Commissiong, Ph.D	Unit Head	NTU, LMCN, NINDS
Others:	Takao Takeshima, M.D., Ph.D	Vis. Fellow	NTU, LMCN, NINDS
	Jane M. Johnston, Ph.D	Vis. Fellow	NTU, LMCN, NINDS
	Helen Balling, B.S.	Biologist	NTU, LMCN, NINDS

COOPERATING UNITS (if any)

Medical Neurology Branch, NINDS

LAB/BRANCH

Laboratory of Molecular and Cellular Neurobiology, BNP

SECTION

Neural Transplantation Unit (NTU)

INSTITUTE AND LOCATION

Park Building, NINDS, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.7	PROFESSIONAL: 2.7	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have established a novel, primary culture of E14 rat ventral mesencephalic dopaminergic neurons, in which, at 12 hr after plating, 95% of the cells are neurons (NSE+), 20% are tyrosine hydroxylase positive (TH+), 5% are glioblasts/neuroblasts (vimentin-positive) and glial fibrillary acidic protein (GFAP) is expressed in <1% of the cells. Dopaminergic neurons in culture exhibit distinct glia independent (at DIV0-DIV9) and glia-dependent (after DIV9) phases of development, even when grown in a medium supplemented with 10% serum. Serum-deprivation caused the selective death of TH+ neurons, while the percentage of GABA-IR neurons increased. These findings, combined with a microisland culturing method, have been used to develop a reliable, sensitive bioassay for dopaminergic neurotrophic factors(DNTFs), which will be crucially important in our purification studies. The lysed extracts from type-1 astrocytes and O-2A progenitor cells, prepared from the ventral mesencephalon, and their conditioned media protect dopaminergic neurons from death. The comparative potency of the protective effect of type-1 astrocytes and O-2A progenitor cells is 1:5. This demonstration of the potent dopaminergic neurotrophic effect of O-2A progenitor cells is a major new finding. We are growing ventral, mesencephalic type-1 astrocytes and O-2A progenitors on a large scale, and will identify, purify and achieve the molecular characterization of at least one DNTF. Preliminary results, based on gel filtration studies using a Sephadex G-75, column indicate that 8 major peaks, all of molecular mass <50 KD, are present in the type-1 astrocytes lysate/conditioned medium, and that significant dopaminergic, neurotrophic activity is retained in peak #7 (fraction #71). Our functional studies demonstrate that Gpla muscle spindle afferents are tightly coupled to their homonymous alpha motoneurons (α-MNs) in spinalized, neonatal, PN7 rats that recover rhythmic stepping in their hindlimbs, but not in spinalized, PN14 rats, which do not recover. Transplantation of a suspension of dopaminergic neurons into the lumbar region of the spinal cord of the PN14 rat, previously spinalized at the middle thoracic level, was associated with functional recovery in the hindlimbs, similar to that seen in the spinalized, PN7 rat. A dense, neodopaminergic innervation of the lumbosacral spinal cord was also observed. We are now uniquely placed to investigate the neurophysiological basis of functional recovery in the mammalian spinal cord that is associated with a dense, dopaminergic reinnervation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01686-25 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Motor Control Systems in the Spinal Cord

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.E. Burke, M.D.	Chief	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	G.M. Dold	Engineering Tech.	LNLC, NINDS
	M.K. Floeter, M.D., Ph.D.	Staff Fellow	LNLC, NINDS
	J.-P. Gossard, Ph.D.	Special Volunteer	LNLC, NINDS
	M. O'Mallely-Davis	Biological Lab. Tech.	LNLC, NINDS

COOPERATING UNITS (if any)

Dept. of Neurosurgery, Children's Hospital National Medical Center, Washgton, DC (Dr. Schiff)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS 3.5 | PROFESSIONAL 2.7 | OTHER 0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The project is designed to provide information about the organization of neuronal systems in the mammalian spinal cord which ultimately controls the activity patterns of motor units (motoneurons and the muscle fibers they innervate). Topics of interest include: analysis of mechanisms of synaptic transmission in the spinal cord, of the reflex pathways within the spinal segment and control of information flow in them by input from primary afferent and supraspinal descending systems, and the organization of synaptic input systems, both segmental and supraspinal, that project to particular motor pools and the interaction of these systems with the spinal mechanisms that generate rhythmic motoneuron output patterns underlying locomotion. Current work concerns the organization of excitatory last-order interneurons in the cat spinal cord, with particular reference to interneurons that transmit short-latency excitation from low-threshold skin afferents and from reticulospinal systems that travel in the medial longitudinal fasciculus (MLF). All these interneuron groups are strongly influenced by the spinal central pattern generator (CPG) for locomotion. The differential patterns of CPG modulation indicate that separate systems of segmental interneurons, each with highly specific patterns of primary afferent and descending convergency, are present in the mammalian spinal cord. We have also studied the sources of variability of motoneuron excitability during monosynaptic reflexes, a subject with specific clinical applications.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01687-25 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Techniques for Making Connections with the Nervous and Musculoskeletal Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.J. Bak	Electronics Engineer	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	F.T. Hambrecht, M.D.	Health Scientist Administrator	DFN, NINDS
	M.J. O'Donovan, M.B.Ch.B.	Section Chief	LNLC, NINDS
	W.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS

COOPERATING UNITS (if any)

Instrumentation and Computer Section, BNP, DIR, NINDS (G.R. Dold)

LAB/BRANCH

Laboratory of Neural Control

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS 0.9 | PROFESSIONAL 0.3 | OTHER 0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is intended to develop techniques and instrumentation for the acquisition and processing of neuroelectric signals from the central and peripheral nervous systems in acute and chronic neurophysiological preparations. Because of this Laboratory's continuing interest in sensorimotor neural activity during unrestrained movements, the project also includes development and fabrication of chronically implantable microelectrodes, mechanical transducers, catheters, and connectors.

Due to the Laboratory's new interests in doing research on isolated preparations such as the spinal cord of chicken embryos, a significant amount of work has been devoted to improving techniques associated with electrical recording, stimulation, and real-time fluorescence microscopy in these preparations. Also included within this report is the development of computer programs of general utility for acquisition and analysis of neuroelectric and mechanical records, as well as of neuroanatomical material.

Several projects which have been associated with the visual prosthesis feasibility studies, normally reported on under this project number, are now being reported under project, Z01 NS 02857-02 LNLC. These projects are referenced as such throughout this report. There are also several other new projects which would normally be listed under this project number but are now listed under the recent visual prosthesis project number.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01688-25 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cortical Mechanisms of Voluntary Motor Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	D. Cole	Biologist	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	W.J. Heetderks, M.D., Ph.D.	Health Scientist Administrator	DFN, NINDS

COOPERATING UNITS (if any)

Fundamental Neurosicence Program, NINDS (W.J. Heetderks); University of Michigan (K. Wise), University of Utah (R. Norman)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Sectio on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: PROFESSIONAL 0.4 OTHER 1.4

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews
- (b) Human tissues
- (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Multicontact passive semiconductor electrodes have been successfully implanted in the arm area of the supplementary motor area (SMA) of a primate that was trained to do a number of different wrist movement tasks. SMA neurons seem to be better correlated with complex tasks than simple repetitive tasks. Recorded activity diminishes in amplitude after several months but can be temporarily restored with microstimulation through the electrode. Neurons in the SMA have been operantly conditioned and they may prove to be a useful signal source of controlling prosthetic devices.

The multicontact silicon electrode developed by the University of Utah, was evaluated for possible incorporation into the studies being conducted by the LNLC. A number of design problems were discovered when the electrodes were implanted at the University of Utah. Further development of the electrode should be left to the University of Utah before we attempt to employ the electrodes at the NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02079-20 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on the line below.)

Models of Neurophysiological Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator on the same title, laboratory, and institute affiliation)

PI:	W.B. Marks, Ph.D.	Research Physiologist	LNLC, NINDS
Others:	R.E. Burke, M.D.	Chief	LNLC, NINDS
	M.J. O'Donovan, M.B.Ch.B., Ph.D.	Research Physiologist	LNLC, NINDS
	T.G. Smith, Ph.D.	Research Physiologist	LNP, NINDS

COOPERATING UNITS (if any)

Lab. of Neurophysiology, NINDS (T.G. Smith); Dept. of Physiol., Yale Medical School, Cambridge, MA (L.B. Cohen)

LAB BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS 1.2 PROFESSIONAL 0.7 OTHER 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In previous Annual Reports we described our approach to summarizing the shape of neuronal dendrites. Measurements from 64 or more reconstructed motoneuron dendrites were interpreted as the result of a hypothetical probabilistic branching process. The probabilities were measured and became parameters for a stochastic algorithm which produced sample dendrites having the statistical properties of observed dendrites. In this report, we describe new methods to predict the average properties of the complete ensemble of these sample dendrites, given the parameters used by the program that produces them. All our methods resemble the stochastic model because they take the configuration of branch diameters at one distance from the stem and transform it into that at the next distance increment. Last year we described a matrix H embodying the model probabilities which generates the probability distributions for branch diameter at all distances. This year this generator has been reduced to a differential equation, so that the properties of our original model and of the structure of dendrites is much clearer. We also have found that the method of generating functions can be used to directly generate the probabilities of complex combinations of branch diameter. This should help us find what properties of motoneurons, which may be complex functions of branch diameter distributions, reach a maximum for the observed values of their shape parameters.

Also we have contributed to an operational definition of "Lacunarity", a fractal measure of shape used by Dr. T. G. Smith to characterize cultured neurons.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02847-01 LENP

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Morphological and Molecular Studies of CGRP in the Gastric Wall

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

Principal Investigator:	G. Jakab, M.D.	Visiting Scientist	LENP, NINDS
Others:	E. Mezey, M.D.,	Visiting Scientist	CNB, NINDS
	K. Pacak, M.D.	Visiting Fellow	CNB, NINDS
	H. deF. Webster, M.D.	Chief	LENP, NINDS

COOPERATING UNITS *(if any)*

LAB BRANCH
Laboratory of Experimental Neuropathology

SECTION
Cellular Neuropathology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to study the distribution of the calcitonin gene-related peptide-immunoreactive (CGRP-IR) nerve fibers and cells in the intact rat gastric mucosa and around an experimental stress ulcer. The CGRP-IR nerve fibers branching pattern suggests the existence of columnar units of innervation that orient perpendicular to the lumen. These units may form the skeleton of a complex vasoregulatory system based on the axon reflex mechanism mediated by neuropeptides. We found a number of CGRP-producing cells (likely neutrophil leukocytes) located mainly in the lower region of the lamina propria. Our observations suggest that the effectivity of the gastric mucosal defense mechanism against chemical provocations can be improved by the involvement of leukocytes committed to produce a variety of mediator compounds. The distribution of leukocytes and their close relationship with peptidergic peripheral nerve fibers suggest that CGRP may play a role in the homing and chemotaxis of circulating immune cells. CGRP can be considered as a trophic factor regulating the gastric microcirculation which is crucial for the maintenance and regeneration of the protective mucosal barrier. Our data confirm the hypothesis that the peripheral nervous system may collaborate with the mobile elements of the immune system associated with various organs and tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02254-17 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Repair of Injured Nervous Tissue with Foreign Grafts

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A. A. Zalewski, M.D.	Section Chief	LNLC, NINDS
Others:	N. A. Azzam, Ph.D.	Special Expert	LNLC, NINDS
	R. N. Azzam	Biologist	LNLC, NINDS
	J. D. Ziemnowicz	NIH Special Volunteer	LNLC, NINDS

COOPERATING UNITS (if any)

CNS Disorders Research, The Upjohn Co., Kalamazoo, MI (L.R. Williams); Transplantation Laboratory, American Red Cross, Rockville, MD (G.M. Fahy)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neuronal Regeneration

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS 3.8 | PROFESSIONAL 2.0 | OTHER 1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

After immunosuppressive therapy with Cyclosporin A (Cy A) is stopped, nerve allograft rejection occurs and host axons that had regenerated into the graft degenerate despite the fact that the axons are not foreign tissue. The present experiment was performed to correlate immune events and cellular loss in nerve allografts after terminating Cy A treatment. Nerve grafts (4 cm long) were taken from American Cancer Institute rats and joined to peroneal nerves of Fischer rats that received Cy A (10 mg/kg, intraperitoneally) for one week. This treatment protocol delays nerve graft rejection for weeks during which time it was expected that host blood vessels would unite with vessels in the allograft, isogeneic nerve fibers would undergo Wallerian degeneration, and host axons would regenerate into allogeneic Schwann cell columns. Nerve allografts were examined 2-6 weeks postoperatively by light and electron microscopy. No immune reaction or destruction of allogeneic cells was found in 2- or 3-week-old grafts, and they were invaded proximally by regenerating host axons. At 4 weeks, the perineurium of each graft became infiltrated by mononuclear cells and was destroyed. In addition, many of the endoneurial blood vessels were occluded and their endothelial cells were missing or degenerating. Despite the immune reaction, Schwann cells remained and myelinated many host axons that had grown 2-3 cm into the grafts. However, at 6 weeks, most allogeneic Schwann cells were absent from all grafts, and no host axons were found. There was also evidence (i.e., masses of condensed chromatin) that Schwann cells were killed by apoptosis. These results demonstrate that there is a sequential rejection of cellular components in nerve allografts and that host axonal degeneration is related to adverse immune and/or metabolic effects on allogeneic Schwann cells. Further studies will try to determine whether lymphocytes or macrophages are responsible for allogeneic cell killing and whether their elimination during the rejection process preserves allogeneic Schwann cells and host axons. Research has continued regarding the cryopreservation of nerves. The goal of these experiments is to establish a bank of human nerves which can be used clinically to repair gaps in injured nerves. As a first step, fresh human nerves were transplanted into immunologically deficient nude rats to determine whether this animal could be used to test the viability of cryopreserved human nerves. Human nerves were joined to peroneal nerves of nude rats and examined 4 and 10 weeks postoperatively. None of the grafts were rejected. Rat axons grew through human Schwann cell tubes and many axons were myelinated. Experiments will be performed to determine whether cryopreserved human nerves survive and conduct regenerating axons after transplantation into nude rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02882-01 LENP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on the line between the borders.)

Viruses as Vectors for Gene Transfer to the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator:	J. R. Martin, M.D.	Medical Officer	LENP, NINDS
Others:	S. Keir, Ph.D.	Visiting Fellow	LENP, NINDS
	W. J. Mitchell, D.V.M., Ph.D.	Sr. Staff Fellow	LENP, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Experimental Neuropathology

SECTION

Cellular Neuropathology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

To develop new treatment strategies for genetic diseases of the nervous system, this project, initiated in FY 1993, aims to use viruses as vectors to transfer genes into nervous system cells in animal models. This requires identification of conditions in which viruses can be introduced into appropriate neural cell populations so that they exhibit long-term genomic persistence and expression of the transferred gene, and cause little or no central nervous system (CNS) injury. Neurotropic herpes simplex virus and human adenovirus vectors will be compared for their ability to fulfill these conditions in animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02788-05 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Neuronal Shape

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C.L. Smith, Ph.D.	Senior Staff Fellow	LNLC, NINDS
Others:	J. Drazba, Ph.D.	IRTA Fellow	LN, NINDS

COOPERATING UNITS (if any)

Albert Einstein University (S. Kahn), Case Western Reserve University (V. Lemmon)

LAB BRANCH

Laboratory of Neural Control

SECTION

Section on Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS 1.0 PROFESSIONAL 1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects (b) Human tissues (c) Neither
  - ☐ (a1) Minors
  - ☐ (a2) Interviews

SUMMARY OF WORK (Use standard paragraphs and do not exceed the space provided.)

This project uses structural methods to study neuronal growth cones with the goal of understanding the molecular mechanisms involved in neurite outgrowth and pathfinding. One initiative focuses on the initial outgrowth of neurites from neuronal cell bodies. Neurite formation by isolated peripheral ganglion neurons from chick embryos was examined by time-lapse microscopy with conventional and laser scanning microscopes. Differential interference contrast optics were used to visualize movements of neuronal cytoplasm, as well as movements of small beads attached to the surface membrane, and interference reflection optics were used to record the concomitant pattern of adhesion to the substrate (polyornithine or laminin). Related changes in the distributions of specific components of the cytoskeleton were detected by immunofluorescence labeling methods. Neurons grown in normal medium were compared with neurons grown in medium containing drugs that disrupt microtubules or actin filaments. The results provide a comprehensive picture of the cytoskeletal movements and substrate interactions that lead to the initiation of neurite outgrowth and suggest a plausible model of the underlying molecular mechanisms. Experiments designed to test this model are in progress.

A second initiative examines the adhesive interactions of growth cones with substrates that support their growth *in vivo*. Retinal axon growth cones from embryonic chicks on substrates consisting of different, naturally-occurring adhesion molecules (laminin, N-CAM, N-cadherin or L1) were visualized with time-lapse interference reflection microscopy to determine the distance between the growth cone and the substrate. Growth cones on all substrates formed transient areas of close apposition to the substrate, but the sizes, distributions and dynamics of these contacts differed. These differences help to explain why growth cones migrate more rapidly on some substrates than others.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02857-02 LNLC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Feasibility Study of an Intracortical Visual Prosthetic Device for the Blind

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.M. Schmidt, Ph.D.	Biological Engineer	LNLC, NINDS
Others:	M.J. Bak	Electronics Engineer	LNLC, NINDS
	G.M. Dold	Engineering Technician	LNLC, NINDS
	A. Reina	Summer Student	LNLC, NINDS

COOPERATING UNITS (if any)

Fundamental Neurosciences Program, NINDS (W.J. Heetderks and F.T. Hambrecht); Surgical Neurology Branch, NINDS (C.V. Kufta); Instrumentation and Computer Section, BNP, DIR, NINDS (B. Smith, Chief)

LAB/BRANCH

Laboratory of Neural Control

SECTION

Section on Neural Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 0.9 | PROFESSIONAL 0.4 | OTHER 0.5

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. As reported last year, a 42-year-old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensations of light called phosphenes. Phosphenes were produced with 34 of the 38 electrodes with currents that were 100 to 1000 times lower than had been reported for surface stimulation of the visual cortex. From the data obtained from the first patient, the design concepts for the next patient-implant have been developed. Four arrays of 32 dual hat pin electrodes will be chronically implanted in the visual cortex for a total of 256 electrodes. A new computer control system with TV camera input and a 256-channel microprocessor-controlled stimulator are under development. A miniature percutaneous connector system has been developed that will contain 64 lead wires. Four of these connectors will be implanted in the next human subject to activate the 256 electrodes. A number of new electrode fabrication techniques have been developed and the resultant electrode arrays and percutaneous connector will be tested in animals prior to the next human implant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS-01442-26 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Permeability of Cellular Layers in the Vertebrate Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Bechara Kachar, M.D.	Visiting Scientist	LNO, NIDCD

COOPERATING UNITS (if any)

Marine Biological Laboratory, Woods Hole, MA, Woo Kuen-Lo, Ph.D., Dept of Anatomy, Morehouse Medical School, Atlanta, GA.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892 Marine Biological Laboratory, Woods Hole, MA

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

How tight junctions might prevent small charged solutes from entering the brain (across the blood-brain barrier) was made clear by our previous model of tight junction structure based on a lipidic backbone. This model has been discussed and elaborated in several publications in recent years. A new study of the gap junctions in murine cells by freeze-fracture and freeze-substitution has been completed showing that these gap junctions show considerable structural diversity, falling into there types. One type is so aberrant from typical gap junction structure as to question its categorization as an electrotonic junction. These findings are in press and this project is expected to be in abeyance through the next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-01881-23 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural Basis of Synaptic Transmission

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas S. Reese, M.D	Chief	LN, NINDS
Others:	Jorge E. Moreira, Ph.D	Visiting Scientist	LN, NINDS
	Katsuyuki Miyaguchi, M.D	Visiting Associate	LN, NINDS

COOPERATING UNITS (if any)
R. Llinas, P.M. Reuss Dept of Physiology and Biophysics, New York Univ Medical Center, NY; G. Benshalom, Dept of Morphology, Ben Gurion Univ Negev, Beer Sheva, Israel.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892 Marine Biological Laboratory, Woods Hole, MA

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project deploys a range of structural techniques to examine normal synaptic structure. These approaches have in common their dependence on rapid freezing and direct visualization of living brain by light microscopical techniques. Up until now this project has been engaged in explorations of various live brain preparations suitable for these purposes. Recently, an isolated whole brain preparation maintained *in vitro* by vascular perfusion as well superfusion with artificial cerebrospinal fluid (CSF) has been evaluated. The ultrastructure of surface samples of isolated brains rapidly excised, quick frozen and freeze substituted served as a benchmark to choose a perfusion fixative yielding realistic images of synaptic structures. It could now be determined to what extent deeper cortical regions of perfused brains remained structurally intact. Throughout 2 hr of perfusion, the morphology of synaptic structures in the isolated brain remained equivalent to the normal brain perfuse-fixed *in situ*. These results provide an excellent method for structural work on the isolated brain, and show that the isolated brain can be used for studies of synaptic structure depending on rapid freeze fixation, and are in agreement with the reported persistence of electrophysiological functions in this preparation. The comprehensive effort to develop methods for making and maintaining organotypic brain cultures continues (see also Project # Z01 NS 02610-10 LN). Up until now it has proven consistently difficult to maintain large pieces of mature brain in culture conditions, but new approaches to this problem are being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02551-12 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and Function of Cytoplasmic Motors*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Thomas S. Reese, M.D.	Chief	LN, NINDS
Others:	Paul E. Gallant, Ph.D	Biologist	LN, NINDS
	Marcelo Hernandez, M.D	Exchange Scientist	LN, NINDS
	S. Brian Andrews, Ph.D	Section Chief	LN, NINDS
	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
	Shahid Khan, Ph.D.	Guest Researcher	LN, NINDS

COOPERATING UNITS (if any)
R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD; B J. Schnapp, Dept.Cell. Mol. Physiol., Harvard Med.Sch., Boston, MA; T. Slater, A. Fein, Dept. Physiol., Northwestern Univ Medical School, Chicago, IL.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892. Marine Biological Laboratory, Woods Hole, MA

TOTAL STAFFYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand the distribution and functions of cytoplasmic motors in the axon of neurons. This information is intended to lead to an understanding, at the molecular level, of axonal transport as well as the cytoplasmic organization in the axon. An important current question is how kinesin and dynein are organized on the organelle surface and its microtubule substrate. Quantitative images of kinesin bound to purified, taxol-stabilized microtubules (as described in Project #Z01-NS-02610-10 LN) have provided the first direct evidence for cross-bridging of microtubules by single kinesins, and suggested that kinesin might also translocate microtubules and have a role in microtubule as well as fast axonal transport. These structural methods are now being applied to compare anterograde, kinesin-powered organelles with retrograde, dynein-powered organelles in order to understand how direction of organelle transport is controlled. Endoplasmic reticulum (ER) is another component of axoplasm which interacts with cytoplasmic motors. We have shown by a new dye injection method in cerebellar neurons from brain slices (see report #Z01 NS02841-03 LN) that the ER makes one continuous system throughout the Purkinje neuron. A similar dye method is also being used to investigate movements of the ER is crayfish axons. The bacterial flagellar motor in E. coli has been studied as another example of a motor system than can switch direction of translocation. We recently discovered a new cytoplasmic component of the flagellar motor thought to be involved in directional switching. Biochemical and structural analyses of this cytoplasmic component is expected to lead to an understanding of the directional switching.

*Formerly: Proteins Involved in Axonal Transport and Structure of Neuronal Cytoplasm

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02873-02 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunocytochemistry of Neuronal Cytoplasm

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jorge E. Moreira, Ph D	Visiting Scientist	LN, NINDS
Others:	Thomas S. Reese, M D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Sven Beushausen, Ph.D	Visiting Associate	LN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892 Marine Biological Laboratory, Woods Hole, MA 02543.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Antibodies to defined domains of the light and heavy chains of the motor protein kinesin (from squid axon) have been used for immunolabeling of freeze substituted squid axoplasm. It was necessary to develop and apply cryogenic methods to prevent displacement of soluble kinesin during tissue processing. Initial results with this new method first applied with a conventional polyclonal antibody to kinesin suggested that kinesins are widely distributed in the cytoplasm but several-fold concentrated around cytoplasmic vesicles. New experiments using polyclonal antibodies against a defined protein fragment of the functional head of the kinesin heavy chain confirmed the previous kinesin location. An increase in gold particles over the cytoplasmic level was also seen around mitochondria, ER cisternae, and microtubules. Sections incubated with a polyclonal antibody against squid neurofilament, failed, as expected, to show a selective distribution around vesicles. Distributions of different kinesins are being explored by antibodies which distinguish different kinesin light chain isoforms. This work is expected to elucidate where different members of the kinesin family are found in the axon, leading to a better understanding of how kinesins actually function in the nerve cell

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02835-02 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Subcellular Organization in Excitable Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Evelyn Ralston, Ph.D.	Special Expert	LN, NINDS
Others:	Stefanie Kaech, Ph.D.	Visiting Fellow	LN, NINDS
	Thorkil Ploug, M.D.	Special Volunteer	EDMN, NIDDK
	Sven Beushausen, Ph.D	Visiting Associate	LN, NINDS
	Bernhard E. Flucher, Ph.D.	Visiting Associate	LN, NINDS
	Thomas S. Reese, M.D.	Chief	LN, NINDS

COOPERATING UNITS (if any)

Herman Gordon, Ph.D., Dept. of Anatomy, University of Arizona, Tucson, AZ.

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to understand how mRNAs, proteins and subcellular organelles are distributed and organized in nerve and muscle cells. In muscle, where cells are multinucleated, the retention of some mRNAs and proteins near the nucleus that produces them seems to contribute to the formation of functional domains. We are examining the role of mRNA lifetime in this process are are using the mouse muscle cell line C2. In this experimental system, we are planning to manipulate the lifetime of specific endogenous mRNAs and of foreign mRNAs introduced by DNA transfection. In parallel, we plan to examine the parameters that contribute to the segregation of specific mRNAs between cell body, axon and dendrites in polarized nerve cells. We are characterizing primary cell cultures and neuronal cell lines that acquire polarity in culture and developing protocols to express foreign genes in these cells. We are also investigating the mechanism of vesicle traffic responsible in muscle for the increase in glucose transport following stimulation by insulin or exercise. It is generally believed that, upon stimulation, the intracellular vesicles carrying the glucose transporter GT4 are translocated to and fuse with the cell membrane. We have been studying the localization of GT4 at high resolution in single fibers of the rat soleus muscle, by a combination of classical and confocal immunofluorescence microscopy and by immunogold electron microscopy. We have developed a protocol for pre-embedding immunogold staining of the fibers with which we have detected an ~10-fold increase in the number of GT4 immunoreactive sites in the membrane of insulin-stimulated fibers. Our results clarify several discrepancies in this field. Since several proteins involved in the traffic of synaptic vesicles have counterparts in vesicles in non-neural cells, we are now searching muscle, both at RNA and protein levels, for expression of synaptic vesicle proteins. Initial results suggest, surprisingly, that two synaptobrevins, hitherto considered neural-specific, are actually expressed in muscle, making a stronger case for a general vesicle traffic mechanism.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02872-02 LN

PERIOD COVERED
Octoberr 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Adhesion in Vertebrate Neural Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Judith A. Drazba, Ph.D.	IRTA Fellow	LN, NINDS
Others:	Carolyn L. Smith, Ph.D.	Senior Staff Fellow	LNC, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Brain Structural Plasticity

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed at characterizing differences in the adhesive patterns and associated growth characteristics of neuronal growth cones on defined substrates. Neurite formation by embryonic retinal ganglion cell neurons was observed using a technique (time-lapse laser scanning interference reflection microscopy) to show local distances of the cell membrane from substrates composed of purified, biologically relevant cell and substrate adhesion molecules. Members of the three major classes of adhesion molecules were tested - the calcium-dependent, calcium-independent, and the immunoglobulin superfamily of molecules. Previously observed differences in the overall degree to which growth cones adhered to different molecular substrates were minimized when we took into account potential variability in our optical method. We, therefore, cannot conclude whether a critical level of attachment is necessary for migration. However, the patterns of attachment on different substrates remained highly distinct even when variability was accounted for. Growth cones on all substrates had some areas of close apposition to the substrate but none of them exhibited dark intensities that were comparable to the focal contacts seen in fibroblasts. Analysis of the dynamic changes in the adhesion patterns also showed wide variation among substrates. Growth cones on L1, for example, exhibited a complicated pattern of close and distant attachment that varied greatly in both temporal and spatial domains. Growth cones on laminin, on the other hand, exhibited a roughly uniform level of distant attachment with a few areas of close attachment at the leading edge. Dynamic changes in adhesion over time and between numerous points on the growth cone were relatively small by comparison. Since growth cones *in vivo* may encounter all of these molecules, and others at the same time, it seems reasonable to suggest that they must be able to integrate these many signals into a response suitable for directing them to their appropriate targets. The question remains why the patterns of adhesion of growth cones to different substrates differ and how those distinct patterns may be involved in the secondary signaling mechanisms that promote and direct neurite outgrowth. Experiments will continue to analyze adhesive interactions with molecules in combination and in patterned arrays. We will also begin to investigate the role of cytoskeletal interactions with adhesion molecules on the cell surface to understand how signals in the growth cone's environment may be transduced into behavioral responses.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02871-02 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Postsynaptic Densities: Mechanisms for Structural Modification

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ayse Dosemeci, Ph.D.	Visiting Associate	LN, NINDS
Others:	Thomas S Reese, M. D.	Chief	LN, NINDS

COOPERATING UNITS (if any)

Howard Jaffee, Ph.D., Protein/Peptide Facility, LNC, NINDS

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Structural changes in postsynaptic densities (PSDs) may underlie long-term modifications of synaptic activity. The aim of this project is to study the molecular organization of the PSDs and to explore the potential mechanisms for their modification in response to calcium and other intracellular messengers. The present research focuses on two calcium-dependent enzymes, calcium calmodulin-dependent protein kinase (CaM kinase) and calpain, as likely candidates for mediating such changes. In order to explore a potential role of calpain-mediated proteolysis in structural modification, PSD preparations were treated with exogenous calpain. Limited calpain action resulted in selective proteolysis of a few proteins including spectrin, while causing little or no breakdown of other proteins, including CaM kinase and actin. Under these conditions, the 160 kDa breakdown product of spectrin cosedimented with the PSDs. Structural changes following calpain treatment were evident by electron microscopy of freeze substituted specimens. Studies on the autophosphorylation of the PSD-associated CaM kinase have continued. Extensive calcium-dependent autophosphorylation of the alpha subunit of the kinase, which is the major protein in densities from cerebral cortex, was obtained by inhibition of the endogenous phosphatase activity as described previously. None of the major proteins of PSDs including CaM kinase itself, nor various subtypes of glutamate receptor subunits, were solubilized following calcium-dependent phosphorylation. Structural modifications due to autophosphorylation are currently being characterized by electron microscopy of freeze substituted PSDs. In collaboration with Dr. Howard Jaffe (LNC-NINDS Protein/Peptide facility) the autophosphorylated sites are being identified by sequencing of tryptic peptides. Similar strategies will be applied to characterize calcium-independent autophosphorylation of PSD-associated CaM kinase. Identification of potential phosphorylation sites of the kinase during and following the calcium signal and determination of correlated structural and biochemical changes in PSDs is expected to help elucidate the role of CaM kinase in synaptic modification.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02841-03 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function of the Endoplasmic Reticulum

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mark Terasaki, Ph.D.	Senior Staff Fellow	LN, NINDS
Others:	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Jorge E. Moreira, Ph.D.	Visiting Scientist	LN, NINDS
	Thomas S. Reese, M.D.	Chief	LN, NINDS

COOPERATING UNITS (if any)

L.A. Jaffe, A. Fein, Univ. Conn. Health Ctr., Farmington, CT; S L. Tamm, Boston Univ. Mar. Prog.; N.T. Slater, Northwest. Univ. Med. Sch.; C. Sardet, CNRS, Villefranche, France

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Structural Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure and function of the endoplasmic reticulum (ER) in neurons and glia are being investigated using newly developed techniques which make it possible to investigate the dynamic properties of the ER in living cells by video and laser scanning confocal microscopy. New techniques have been developed using sea urchin eggs as a model system. Sea urchin eggs have the advantages of being readily available, having a prominent ER, and being easy to microinject. A novel technique for specifically staining ER in living cells shows that the ER undergoes actin-dependent movements, that a striking change in its organization occurs as it becomes capable of releasing calcium at the time of calcium release, and that the appearance of microtubules has a profound effect on its organization. This technique has been applied to Purkinje neurons of the cerebellum in acute slices, where it shows that there is a continuous compartment of ER that extends from the cell body throughout the dendritic tree. The structure and dynamics of the ER is currently being investigated in hippocampal neurons in culture, where the ER may have a role in the establishment of axonal and dendritic polarity. A second initiative uses calcium-sensitive fluorescent indicators to investigate calcium regulation by the ER. Calcium increase during ciliary reversal has been detected, and calcium regulation in hippocampal neurons in culture is being investigated with these techniques A third initiative seeks to determine the distribution of noncortical actin filaments in neurons using a novel technique.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02842-03 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Molecular Biology of Neural Function *

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sven A. Beushausen, Ph D	Visiting Associate	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Delia Tang, M.D	IRTA Fellow	LN, NINDS
	Howard Jaffe, Ph.D.	Special Expert	LNC, NINDS
	K. Tarananth Shetty, Ph D	Visiting Scientist	LNC, NINDS
	Harish Pant, Ph. D	Research Chemist	LNC, NINDS
	Joanne Gutierrez, B S	Chemist	LCB, NIMH

COOPERATING UNITS (if any)

H. Bayley, Worcester Found Exp Biol, Shrewsbery, MA; M. Miller, K. Weiss, W. Probst, Dept Phsyiol & Biophys., Mt Sinai Sch Med, NY. G. Chin, Science Mag, G. Augustine, Dept Neurobiol. Duke Univ, NC.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Structural Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The following summary describes five projects that attempt to investigate, at the molecular level, the roles a number of molecules, including, A kinase, kinesin light chains, the modulatory neuropeptides buccalin and myomodulin, rab 3 and a novel protein thought to regulate a cdc-2-like protein found exclusively in neurons, play in neural function. We have characterized the peptide families of buccalin and myomodulin from the sea hare *Aplysia californica* in an attempt to gain further insights as to how peptide families collectively contribute to pre- or postsynaptic neuromodulation. The molecular characterization of peptide receptors through which signal transduction cascades are activated to affect modulation, for example G protein-coupled receptors, and their respective regulation by A kinases is also being actively pursued. A family of kinesin light chain transcripts have been identified in the nervous system of the squid *Loligo pealei*. Protein over-expression and purification is currently being employed as a means to determine function and specificity as they relate to intracellular transport, particularly axonal transport of membrane bound organelles in neurons. The small, synaptic vesicle-specific, GTP-binding protein, rab 3a, has been cloned from the squid optic lobe. Microinjection experiments utilizing either whole protein or fragments of over-expressed rab 3a, and rab 3a peptide-specific antibodies will be used to help determine the role rab 3a plays in synaptic vesicle docking and or fusion at the presynaptic membrane. A fifth protein, p62, thought to regulate the activity of a neurofilament-specific cdc-2 protein kinase-like activity has been cloned and characterized from a rat brain cDNA library.

*Formerly entitled "Catalytic Subunit Characterization of the cyclic AMP-Dependent Protein Kinases from the Marine Mollusc *Aplysia californica*" and "Molecular Biology of Neural Function in Invertebrate Models".

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02610-10 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Elemental and Structural Organization of Neurons and Glia*

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. Brian Andrews, Ph.D	Section Chief	LN, NINDS
Others:	Thomas S Reese, M.D	Chief	LN, NINDS
	Roger Buchanan, Ph.D.	IRTA Fellow	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS *(if any)*
R.D Leapman, BEIP, NCCR, NIH, Bethesda, MD D M.D Landis, Case-Western Reserve Univ, Cleveland, OH B D Trapp, Johns Hopkins Univ Sch Med, Baltimore, MD R.A Buchanan, Arkansas State Univ, State College, AK J A. Connor, Roche Inst, Nutley, NJ

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Analytical Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.6	0.2

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This two-part project studies the organization and function of specialized membranes in neurons and glia. The first part aims to characterize calcium regulation during synaptic activity in parallel fiber/Purkinje cell synapses of the cerebellar cortex and in synapses of CA3 hippocampal pyramidal cells. New frozen sectioning techniques, combined with scanning transmission electron microscopy (STEM), have permitted studies of coordinated changes in cytoplasmic total calcium which accompany the regulation of free intracellular calcium by endoplasmic reticulum. A new method, based on darkfield mass mapping in the STEM, has been used to determine the *in situ* molecular mass of organelles within neuronal processes. Such measurements have provided fundamental new information on the effects of beam-induced mass loss, and have led to new approaches to correcting concentration measurements for such effects. Structural analysis of chemically fixed and directly frozen preparations of a new kind of organotypic culture of hippocampus has identified culture conditions which provide excellent organization of CA3 mossy fiber synapses. These synapses are close enough to the surface to be suitable for direct freezing studies. In the second part, the assembly of specialized myelin membranes is studied. Confocal light microscopy had previously shown that Schwann cells depend on microtubules for intracellular transport and assembly of myelin-specific proteins. Now, we have found that the organization of the Schwann cell microtubule network and therefore also of the organelles and filaments of cytoplasmic channels which depend on microtubules for their organization is obligatorily depend on axonal contact. Thus, the characteristic polarization of Schwann cell surface membranes does not occur in the absence of axons, and the proper sorting and targeting of myelin proteins consequently cannot take place.
*Formerly: "Distribution of Mobile and Structural Components at Chemical Synapses"

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02836-03 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structural and Elemental Analysis of Macromolecular Assemblies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Brian Andrews, Ph.D.	Section Chief	LN, NINDS
Others:	Thomas S. Reese, M.D.	Chief	LN, NINDS
	Paul E. Gallant, Ph.D.	Biologist	LN, NINDS
	Maureen F. O'Connell, B.S.	Biologist	LN, NINDS

COOPERATING UNITS (if any)
R.D. Leapman, BEIP, NCCR, NIH, Bethesda, MD. J.A. Hunt, Lehigh University, Bethlehem, PA.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Analytical Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892; Marine Biological Laboratory, Woods Hole, MA 02543

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to characterize the shape, molecular weight distribution and elemental composition of individual macromolecules and macromolecular assemblies. Such assemblies are critical to many cell functions, and their behavior in vitro reflects their function and regulation in intact cells. This project depends on a unique instrument a low-temperature, high-resolution, field-emission scanning transmission electron microscope (STEM) for molecular weight mapping and chemical analysis by parallel electron energy loss spectroscopy (PEELS) of directly frozen thin films and ultrathin cryosections of directly frozen tissues. Dark-field molecular weight mapping of squid brain kinesin has revealed a new conformation of this motor protein, in which the kinesin light chain end is folded back onto the stalk of the molecule near its hinge; this results in an apparently shortened stalk region. The potential significance of this conformation is suggested by mass analysis of kinesin/microtubule complexes, where we have found that single kinesins can crossbridge microtubule pairs with a typical spacing of 25 nm, consistent only with the shortened conformation of kinesin. The ability of single kinesins to crossbridge microtubules implies a second, previously unrecognized, microtubule binding site on the light-chain end of kinesin, and suggests that kinesin may play a role in stabilization of microtubule arrays and in microtubule sliding. We have applied a new method based on analyzing the valence electron region of a low-dose PEELS map of frozen-hydrated sections to determine the optimal thickness of cryosections for PEELS elemental analysis and to measure the distribution of water within Purkinje cell dendrites. This method in combination with high-dose PEELS spectrum imaging has been used to measure calcium in Purkinje dendrites with a fourfold improvement in sensitivity.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02834-03 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Development of Excitation-Contraction Coupling in Muscle

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Bernhard E. Flucher, Ph.D	Visiting Associate	LN, NINDS
Others:	S. Brian Andrews, Ph.D	Section Chief	LN, NINDS
	Maureen O'Connell, B.S	Biologist	LN, NINDS

COOPERATING UNITS (if any)
M.P. Daniels, LGB, NHLBI, NIH, Bethesda, MD; J.A. Powell, Smith College, Northampton, MA; C. Franzini-Armstrong, Penn Philadelphia, PA; K. Beam, Colorado State, Fort Collins, CO; V.M. Fowler, Scripps, La Jolla, CA, M.A. Sussmann, UCLA, Los Angeles,

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Analytical Cell Biology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to determine the molecular mechanisms involved in the assembly of the triad junction between T-tubules and sarcoplasmic reticulum during the development of excitation-contraction (E-C) coupling in skeletal muscle. Immunofluorescence studies of the distribution of the skeletal muscle dihydropyridine receptor (DHPR)(the putative voltage sensor in E-C coupling), the ryanodine receptor (RyR) (the calcium release channel of the sarcoplasmic reticulum) and triadin in developing normal muscle and dysgenic (mdg) myotubes in culture showed that a protein-protein interaction mediated by the DHPR play a role in the normal organization of the triad proteins. The α1 subunit of the DHPR is essential for the normal targeting of the α2 subunit; it also facilitates the normal organization of the RyR and triadin although it is not absolutely required. *De novo* expression of the DHPR α1 subunit from normal nonmuscle nuclei fused with dysgenic myotubes restored normal functions and normal molecular organization of the E-C coupling membranes. Recordings of cytoplasmic free calcium with fluorescent indicators revealed three types of calcium transients in developing myotubes: action potential-induced transients, fast localized transients, and propagated calcium waves with at least two underlying mechanisms: E-C coupling and calcium-induced calcium release. Only action potential-induced transients are eliminated in the dysgenic mutant, suggesting that fast localized transient and calcium waves represent properties of the RyR independent from interactions with the DHPR.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-01805-25 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Membrane Structure of Astrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Milton W Brightman, Ph D.	Section Chief	LN, NINDS
Others:	Elena Sanovich, Ph.D.	Special Volunteer	LN, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is being held in abeyance until fiscal year 1994.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02086-20 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regeneration Specificity in Transplanted Neural Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	David L. Simpson, M.D.	Special Expert	LN, NINDS
Others:	Milton W. Brightman, Ph D	Section Chief	LN, NINDS

COOPERATING UNITS *(if any)*
Jung-Hwa Tao-Cheng, Ph.D, EM Facility, NINDS, NIH; J. Bressler, Ph.D., Kennedy Institute, Baltimore, MD. Richard Burry, Ph.D., Ohio State University, Columbus, OH.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Brain Structural Plasticity

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The neuronal differentiation of PC12 cells is being further defined in three ways: (1) the *in vitro* effect of brain cells on the neuronal differentiation of PC12 cells, treated with nerve growth factor (NGF) or infected with ras-oncogene, that might account for the survival of PC-12 cells grafted to brain. Specifically ,the expression of choline acetyl transferase (ChAT) and acetylcholine esterase (AChE) are assayed biochemically. Structural changes are assessed by electron microscopy. PC12 cells, differentiated either by NGF or ras-oncogene, are cocultured with astrocytes or brain endothelial cells. After 6-14 days in coculture, the number of neurites, their varicosities and clusters of synaptic vesicles, identified immunocytochemically by their content of synapsin and synaptophysin, all increase over PC12 cells in solo culture As controls, fibroblasts were used instead of astroglia and bovine aortic endothelium instead of brain endothelium The release of ^{32}P-dopamine after K+ stimulation is 3-5 times greater from ras-PC12 cells than from naive cells in solo culture. (2) As a major component of cell surface adhesion molecules that may affect the association of PC12 cells with brain cells, sialic acid, measured by HPLC, decreases after NGF treatment but rises markedly in oncogene treated PC-12 cells (3)The signaling pathway for the expression of growth-associated protein (GAP)-43 induced by NGF and by ras-oncogene are being compared. The time course for the expression of GAP-43 and the augmented neurite outgrowth were similar in cells treated with NGF or with ras-oncogene. Both events appear to be signaled through a pathway involving activation of the regulatory G protein, p21, encoded by the oncogene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02869-02 LN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Influence of Leukocytes on Neural Regrowth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Shigeru Naito, M.D.	Visiting Fellow	LN, NINDS
Others: Lisa	Chang, B.S	Biologist	LN, NINDS
	Nicholas DiProspero, B.S	Summer IRTA	LN, NINDS
	Milton W. Brightman, Ph.D	Section Chief	LN, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION

Section on Brain Structural Plasticity

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFFYEARS: 1.1	PROFESSIONAL: 0.6	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to determine whether axons of a damaged spinal cord can be induced to regenerate by the administration of exogenous macrophages (MØ). The MØ are activated by lipopolysaccharide (LPS) or phytohemaglutinin (PHA), with the intent of promoting their secretion of growth factors over that of proteases. The exogenous, isogeneic MØ should provide growth factors that might promote axonal regeneration across the lesion. The spinal cord of adult rats is crushed epidurally, at the T-7 segment of rats, by compressing the cord between the blades of a fine forceps. The blades are separated by an adjustable distance. The activated MØ are labeled with a lipid soluble, fluorescent dye and are now injected intravenously at different times over a 1 to 4 week period following the injury. In other injured rats, the MØ are injected, first, directly into the cord at the lesion site and then intravascularly thereafter. The exogenous, activated MØ are attracted to and accumulate at the injured site. As the pathological changes in damaged cords are variable so are the number and position of intact axons. Consequently, spared axons must be distinguished from regrowing ones. There is now a method, the immunostaining of GAP-43 with antibodies at high dilution, which, in preliminary findings, appears to distinguish normal from regenerating axons. To date, only a few such axons are discernible. The next steps are to find the reagents that optimally release the MØ growth factors and to identify the cytokines that may also be secreted by these MØ and which may be prime effectors of tissue repair.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01-NS-02144-19 LN

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Blood-Brain Barrier

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Shigeru Naito, M.D.	Visiting Fellow	LN, NINDS
Others:	Elena Sanovich, Ph.D	Special Volunteer	LN, NINDS
	Lisa Chang, B S	Biologist	LN, NINDS
	Milton W. Brightman, Ph D	Section Chief	LN, NINDS

COOPERATING UNITS (if any)
Susan Doctrow, Ph D., Senior Research Scientist, Alkermes, Inc. Boston, MA.

LAB/BRANCH
Laboratory of Neurobiology, BNP, DIR, NINDS

SECTION
Section on Brain Structural Plasticity

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.2	0.7	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The hypothesis that a blood vessel's phenotype is determined by the target tissue being vascularized rather than by the source of the vessel is being tested further because it does not apply to mature tissue. When an autograft of mature skeletal muscle is vascularized by the fenestrated vessels of mature choroid plexus, the fenestrated phenotype is retained rather than being replaced by the muscle type. This exception may be due to the mature muscle graft being incapable of secreting a conversion factor or to the mature, ingrowing vessels being unresponsive to such a factor. To test this hypothesis, 13 - 14 day old fetal muscle, and choroid plexus still attached to the brain stem, are being cografted to the IV ventricle and cerebral or cerebellar cortex of adult rats. Fetal tissue is identified by its bromodeoxyuridine that had been injected into the pregnant rat. Although fetal muscle survives when grafted to the IV ventricle, very few of the grafted fetal choroid plexuses do. Cografts to the cerebral or cerebellar cortex are being attempted for better choroidal survivability and to differentiate donor from host choroid plexus without the need for a label.

The mechanism of opening the blood-brain barrier (BBB) with RMP-7, a bradykinin analog, is also being studied. RMP-7 presumably opens the BBB only to small molecules such as sucrose and inulin (MW 5,000), by binding to a receptor on the luminal side of the endothelium. Attempts have begun to learn whether probe molecules such as biotinylated or fluorescein-conjugated dextran, MW 3,000, visible by light and electron microscopy, pass the barrier by receptor-mediated transcytosis initiated by the RMP-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-00813-32 LNC

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Enzymological Aspects of Neural Functions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	R. Wayne Albers, Ph D	Section Head	LNC, NINDS
Others:	William T. Link, Ph. D	Senior Staff Fellow	LNC, NINDS
	Alexander Wheaton	Biologist Lab Technician	LNC, NINDS

COOPERATING UNITS (if any)

J.P. Froehlich, Ph D , M.D , NIA, NIH, Baltimore
K. Fendler, Max-Planck-Institut fur Biophysik, Frankfurt, FRG

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Section on Enzyme Chemistry

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is comprised of research into the structure and functioning of ion transport systems. There are currently three active subprojects:

1) Transient kinetics: A collaborative study with Froehlich and Fendler on the source of the transmembrane current that is generated by phosphorylation of the sodium pump has been completed. Collaboration with Froehlich on the source of the biphasic characteristics of phosphorylation and dephosphorylation is continuing.

2) Investigation of posttranslational modifications of the sodium pump. This project involves characterization of identified fragments of the sodium pump catalytic subunit by mass spectrometry.

3) Studies of the effects of kinase and phosphatase inhibitors and activators on sodium pump activity. These are studies carried out on preparations that will be correlated with the work in subproject # 2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02723-07 LNC

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peptides in the Adult and Developing Vertebrate Nervous Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harold Gainer, Ph.D	Chief	LNC, NINDS
Co-PI:	Susan Wray, Ph.D	Research Cell Biologist	LNC, NINDS
Others:	Sharon Key, B.S.	Biologist	LNC, NINDS
	Kiyoshi Kusano, Ph.D.	Visiting Scientist	LNC, NINDS
	Christopher Flores, Ph.D	PRAT Fellow	LNC, NINDS
	Susan Bachus, Ph.D	IRTA Fellow	LNC, NINDS
	Diane Witt, Ph.D	IRTA Fellow	LNC, NINDS

COOPERATING UNITS (if any)

Dr. M. Castel, Hebrew University, Israel; Dr. M. Morris, Wake-Forest University, Winston-Salem, NC

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.5	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to elucidate the mechanisms underlying the developmental and homeostatic regulation of gene expression in luteinizing hormone releasing hormone (LHRH) and magnocellular oxytocin (OT) and vasopressin (VP) neurons in the hypothalamus, and substance P and calcitonin gene-related peptide (CGRP)-synthesizing sensory neurons in peripheral ganglia

We have used a slice-explant tissue culture system which maintains differentiated LHRH- and OT-neurons for long periods of time *in vitro*, to study the effects of potassium depolarization and second messenger activation on neuropeptide gene expression. Assays of mRNA levels in single cells was done by quantitative *in situ* hybridization histochemistry and image analysis. We found that 40 mM K^+ increased OT mRNA levels two-fold, but had no observable effect on LHRH mRNA levels. Both cell types, however, responded to this stimulus by increased c-fos expression. Forskolin treatment resulted in an increase in neuropeptide mRNA in both OT and LHRH neurons after an 8 hr exposure. Experiments to evaluate the turnover rates of LHRH and OT mRNA in these cultures utilized actinomycin D to inhibit transcription. The rate of decay of mRNA after this treatment suggested a relatively fast turnover for LHRH mRNA ($t_{\frac{1}{2}}$ <24 hr), and a much longer turnover rate ($t_{\frac{1}{2}} \geq 48$ hr) for OT mRNA. Studies of the suprachiasmatic nucleus *in vitro* showed VP expressing cells exhibited developmental changes (increased levels of expression) similar to that seen *in vivo*. Numerous vasoactive intestinal polypeptide cells were detected in these explants while gastrin releasing polypeptide, known to be robustly coexpressed in these same cells at later postnatal times *in vivo* showed low level expression independent of culturing time. GABAergic cells comprised a prominent subtype in these explants and electron microscopic analysis revealed many internuclear synapses. We also found that culturing slices on a permeable membrane, Anocell, dramatically increases survival of OT neurons *in vitro*. Estrogen receptor (E_2 R) mRNA probes were validated in uterus and pituitary *in vivo*, and used to show that OT cells do not contain E_2R. We have characterized developmental expression patterns of substance P and CGRP in neurons in the rat trigeminal ganglion (TGG) *in vivo*, showing that both peptides reach maximal levels of expression in adult animals. In addition, we have begun to examine the enzymes implicated in the posttranslational modification of neuropeptides into their biologically active forms. The content of mRNA encoding carboxypeptidase E, peptidylglycyl-α-amidating monooxygenase, prohormone convertase 1 and prohormone convertase 2 was studied. Preliminary results indicate that each of these candidate neuropeptide processing enzymes is expressed in virtually all neurons in the TGG, and does not appear to be selectively expressed in known peptidergic neurons in the ganglion

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02724-07 LNC

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms in Neuronal Structure and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Harold Gainer, Ph.D	Chief	LNC, NINDS
Co-PI:	Harish C. Pant, Ph.D.	Research Chemist	LNC, NINDS
Others:	Margi Goldstein, Ph.D	Senior Staff Fellow	LNC, NINDS
	Shirley B. House, B.S.	Biologist	LNC, NINDS
	Christopher Flores, Ph.D	PRAT Fellow	LNC, NINDS
	Philip Grant, Ph.D.	Special Expert	LNC, NINDS

COOPERATING UNITS (if any)

A. Giuditta, Ph.D., Institute of Biophysics, Naples, Italy; M. Tytell, Ph.D., Wake-Forest University, Durham, NC, D.B. Henken, LENP, NINDS

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.9	PROFESSIONAL: 2.2	OTHER: 0.7

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The primary goal of this project is to study the gene expression, metabolism, and functions of neuronal intermediate filament proteins (e.g., neurofilament (NF) proteins) in the developing and adult nervous system. For this purpose, we have characterized sensory neuron tissue cultures derived from fetal (E15-E20) and postnatal (>PN2) dorsal root (DRG) and trigeminal ganglia (TGG). The neurons in these ganglia were analyzed for their expression of seven target genes *in vivo* and *in vitro*. These included neurofilament-L, -M, -H, peripherin, α-tubulin, calcitonin gene-related peptide (CGRP) and substance P. Analysis for mRNA was done by Northern blot and *in situ* hybridization histochemistry, and of proteins and peptides by Western blot and immunocytochemistry. The results show robust expression of all seven target genes *in vivo* and in culture. We found that the TGG, like the DRG, contains two major cell types, distinguishable by their size and intermediate filament subtype expression: a population of relatively large cells that express NF-L (61%) and a population of relatively smaller cells that expresses peripherin (35%) with approximately 5% of cells coexpressing both proteins. *In situ* hybridization studies of embryonic DRG show that during development, NF-L mRNA and protein are up-regulated while peripherin protein is down-regulated in the NF-L- immunoreactive (IR) population. Peripherin mRNA remains at high levels in this population throughout development into the adult, despite the lack of peripherin immunoreactivity. We have also developed and characterized an *in vitro* system of DRG neurons to study the regulation of the genes of interest in response to growth factors and target tissues. DRG neurons were cultured from E15, 17, 20 and PN2 rats in the presence of NGF to examine the expression of the above genes by *in situ* hybridization. NF-L and peripherin mRNAs are expressed in every neuron of the E15 and E17 cultures. Between E20 and PN2, peripherin mRNA expression persists, while the number of neurons expressing NF-L mRNA is drastically reduced. E15 cultures treated with skeletal muscle extract show increased levels of NF-L mRNA. These results suggest that factors in addition to NGF are present in skeletal muscle extract and may allow the NF-L phenotype class to be expressed *in vitro*.

In a second project we initiated studies to test the hypothesis that axonal protein synthesis occurs in the squid giant axon. Protein biosynthesis/immunoprecipitation experiments confirmed robust biosynthesis of NF proteins in squid stellate ganglia. Although, we found squid NF protein mRNA in axoplasm, we failed to detect any NF biosynthesis in the giant axon.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1-NS-02725-07 LNC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Phosphorylation and Regulation of Cytoskeleton in Neuronal Systems

P.I.:	Harish C. Pant, Ph.D.	Research Chemist	LNC, NINDS
Others:	Eytan Elhanany, Ph.D.	Visting Scientist	LNC, NINDS
	Howard Jaffe, Ph.D.	Special Expert	LNC, NINDS
	William T. Link, Ph.D.	Senior Staff Fellow	LNC, NINDS
	Kurudunje T. Shetty, Ph.D.	Visiting Scientist	LNC, NINDS
	Veeranna, Ph.D.	Visiting Fellow	LNC, NINDS
	Alexander Wheaton	Biologist Lab Technician	LNC, NINDS

COOPERATING UNITS (if any)

Dr. James F. Battey, LBC, NCI; Dr. Mark R. Hellmich, James M. Way, Biologist, LBC, NCI; Dr. S. Beushausen, LN, NIH, NINDS

LAB/BRANCH

Neurochemistry, BNP, DIR, NINDS

SECTION

Enzyme Chemistry

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 6.0	PROFESSIONAL: 5.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Our progress to understand the structure and function of neurofilaments, (NFs), their phosphorylation and to identify the specific kinases and phosphatases involved is as follows: (1) We have shown that second messenger-dependent protein kinases (PK) phosphorylate the head domains and second messenger-independent PKs, casein kinase I and II and microtubule-associated PK-like activities associated with neurofilament preparation phosphorylate serine residues in the C-terminal tail domain of neurofilament proteins (NFPs' *in vitro* but not the multiple repeat lys-ser-pro (KSP) motifs in middle (NF-M) and high (NF-H) NFPs. (2) The analysis of the phosphorylation state of KSP repeats by means of a combination of chemical and enzymatic digestion, reverse phase high-pressure chromatography, Edman microsequencing and electrospray mass spectrometry showed that most of the KSP motifs in NF-H are phosphorylated *in vivo*; and that the domain containing uninterrupted KSP repeats is highly resistant to proteolysis and can be proteolysed after dephosphorylation. (3) We have identified and isolated from rat spinal cord a protein kinase that phosphorylates a specific KSP sequence (KSPXK) in NF-M and NF-H. Characterization of this enzyme revealed a close relationship to the cell-cycle dependent kinases (CDK), most closely to CDK5. Purification of this CDK5-like kinase from rat spinal cord has shown that it is strongly associated with a protein of 62 kDa (p62). Separation of this protein from the kinase resulted in a considerable decrease in this kinase activity which could be restored by adding back the purified p62. The complete amino acid sequence of p62 was deduced from a number of cDNA clones from rat brain libraries. No similarity exists with any known protein in the current protein sequence data banks. Riboprobe *in situ* hybridization experiments of mouse embryos and adults have demonstrated that p62 transcript expression begins early in development and is restricted to the nervous system, exclusively expressed in neurons and absent from surrounding glia. These studies suggest that CDK-like kinases and related regulators (p62) are involved in phosphorylation of KSP sites in NF-M and NF-H, and may be involved in neuronal growth and differentiation as well as in the stability of axonal structures. (4) We have also demonstrated that the phosphorylation of NF-H by CDK5-like kinase is dephosphorylated by protein phosphatase 2A, and identified such a phosphatase in the rat spinal cord.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1-NS-02757-06 LNC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Studies of Peptidergic Neurons and Peptide Receptors

P.I.:	Kiyoshi Kusano, Ph D	Visiting Scientist	LNC, NINDS
Others:	Harold Gainer, Ph.D	Laboratory Chief	LNC, NINDS
	Susan Wray, Ph.D	Research Biologist	LNC, NINDS
	Susan Fueshko, Ph.D	IRTA Fellow	LNC, NINDS
	Shirley House, B S.	Biologist	LNC, NINDS

COOPERATING UNITS (if any)

Dr. James F. Battey, LBC, NCI

LAB BRANCH

Neurochemistry, BNP, DIR, NINDS

SECTION

Cellular and Developmental Neurobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.4	PROFESSIONAL: 1.1	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Electrophysiological properties of embryonic luteinizing hormone-releasing hormone (LHRH) containing neurons were studied. Olfactory placodes from mouse embryos (E12.5) were dissected and cultured on glass coverslips for 2-3 weeks in defined medium. LHRH neurons emerged from the olfactory placode explants by day 6 in culture together with other olfactory neurons and non-neuronal cells. Electrophysiological recordings were carried out on unidentified neurons by employing whole-cell patch pipettes which contained various intracellular solutions and an additional fluroescent vital dye Lucifer Yellow. Both voltage- and current-clamp techniques were employed. Following the electrophysiological recordings, the cells which had been labelled with Lucifer Yellow, were processed for immunocytochemical identification of LHRH. Fifteen neurons were positively identified as LHRH-containing neurons. These neurons displayed spontaneous spike discharges which were either generated intrinsically or transsynaptically. The somatic region of these cells expressed voltage-senstitive sodium current (I_{Na}), potassium currents (I_A,I_K), and $GABA_A$- receptors. Non-LHRH containing, glutamic acid decarboxylase (GAD), and GABA-containing neurons in these explant cultures were also studied.

Electrophysiological properties of mouse fibroblasts (Balb/C3T3) transfected with cDNA encoding either gastrin-releasing peptide (GRP) or neuromedin B (NmB)-receptors were examined. Both receptors were expressed abundantly and upon activation of these receptors all the transfected cells responded with Ca^{2+} activated K^+-conductance increases. Among various GRP/Bombesin receptor antagonists examined, [D-Phe^6]-Bn(6-13) ethyl ester was the most effective in suppressing GRP-receptor activities. In general, the GRP antagonists were much less effective on NmB-receptors. Using a calcium imaging/photometry system, we have also shown that these peptides evoked intracellular Ca^{2+} increases in these transfected fibroblasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1-NS-02820-04 LNC

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cloning and Functional Analysis of Genes Active in Neurogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

P.I.:	Ward F Odenwald, Ph D	Senior Staff Fellow	LNC, NINDS
Others:	Ravi Kambadur, Ph D	Visiting Associate	LNC, NINDS
	Shang Ding Zhang, M D	Visiting Associate	LNC, NINDS
	Peter Vos, Ph D	IRTA Fellow	LNC, NINDS

COOPERATING UNITS (if any)

B A Olde, Ph.D., NG, NINDS

LAB/BRANCH

Neurochemistry, BNP, DIR, NINDS

SECTION

Cellular and Developmental Neurobiology (Neurogenetics Unit)

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
4.0	4.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this program is to identify and functionally characterize neurogenic genes that are required for CNS development. Given the high conservation in basic mechanisms used by all metazoans, our search was initiated in the fruit fly (*Drosophila melanogaster*) where the neurogenic genes are more accessible for study. Utilizing classical genetic, molecular biology and transgenic techniques, we have continued to study both the function and the regulatory mechanisms that control the expression of *castor*, a novel Zinc finger gene required for *Drosophila* CNS development and *pollux*, *castor's* close genomic neighbor. Based on its predicted primary structure and the high expression levels in CNS neuroblasts, *castor* may regulate itself and other genes involved in neuroblast maturation. To test this hypothesis, we are currently mapping the *cis*-regulatory elements that control its *in vivo* expression. Located in its 5' flank, we have found a near perfect 880bp inverted repeat and are now determining if it harbors *cis*-elements that regulate expression. Once identified, we will assess if lack of or ectopic *castor* expression modulates reporters that respond to these *cis*-elements. We are also determining if the *pollux* protein functions as a membrane-associated adhesion molecule. Analysis of its primary structure reveals that *pollux* contains an integrin-binding tetrapeptide RGD sequence, multiple glycosaminoglycan-binding sites, and a potential glycosaminoglycan-linkage site. *pollux* immunostainings have shown that a portion or all of the protein is located on the plasma membrane extracellular surface. In addition, we have observed that misexpression of *pollux* leads to high levels of mature male homosexual activity. Protein data bank searches, have revealed that *pollux* shares a 70 amino acid domain with the human tre-1 oncogene and a predicted human myoblast protein (84% and 87% similarity).

We have also continued our functional analysis of the murine homeobox gene *Hox* 1.3 by identifying genes that it regulates. We have observed that ectopic expression of *Hox* 1.3 in transgenic mice correlates with the apparent repression of a hepatocyte nuclear transcription factor, HNF-3β. During development, we have also discovered that HNF-3β is expressed in the CNS and are now assessing if *in utero* ectopic *Hox* 1.3 expression modulates its expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS 02528-03 LNC

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Analysis of Calcium Channel Gene Expression in Mammalian Nervous System

PI:	Hemin R. Chin, Ph.D.	Research Cell Biologist	LNC, NINDS
Others:	[illegible] Jung, Ph.D.	Visiting Fellow	LNC, NINDS
	Chi-[illegible] Kwon, M.D., Ph.D.	Visiting Fellow	LNC, NINDS
	[illegible] Hwang, M.D., Ph.D.	Special Volunteer	LNC, NINDS

COOPERATING UNITS (if any)

[illegible], M.D., Ph.D., University of Michigan School of Medicine

LAB/BRANCH

Neurochemistry, BNP, DIR, NINDS

SECTION

Section on Molecular Neuroscience

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.5 PROFESSIONAL: 3.5 OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

SUMMARY OF WORK: The long term goals of this project are to: 1) understand the molecular basis for the structure-function and tissue-specific expression of voltage-sensitive calcium channels (VSCC) and 2) elucidate the functional roles and expression patterns of VSCC during development. Multiple types of VSCC (i.e., T, N, L and P-type) are present in both excitable and nonexcitable cells. They are important for excitation-contraction coupling, secretion of neurotransmitters and hormones, and regulation of gene expression. During the past year, we have developed several molecular reagents useful for analysis of calcium channels. They include: 1) brain cDNAs corresponding to the α1 subunits of the dihydropyridine (DHP)-sensitive L-type as well as DHP-insensitive N and P-type calcium channels; 2) cDNA corresponding to the α2 and β subunits of calcium channels; and 3) polyclonal antisera specific for each type of VSCC subunit proteins. In situ hybridization histochemistry and immunocytochemistry techniques were utilized to examine spatiotemporal expression patterns of multiple VSCC subunit mRNAs and proteins in adult and developing rat brains. Our results have indicated that heterogeneous types of VSCC are expressed at an early embryonic stage (E12), suggesting that they may play important roles even in developing central nervous system (CNS). Ongoing analysis of genetic heterogeneity of various VSCCs expressed during CNS development are in progress. In addition, Northern blot hybridization analysis revealed that L-type VSCC mRNA was present in a number of human tissues examined including brain, heart, lung, kidney, pancreas and skeletal muscle, whereas the N-type VSCC transcript was exclusively present in neuronal tissues. As a first step toward understanding the genetic bases for the tissue-specific expression of VSCCs, we have isolated the human genomic clones for the L and N-type calcium channel α1 subunits. The 5' flanking promoter regions of the L-type and N-type VSCCs will be analyzed to identify and characterize important regulatory elements that either enhance or repress expression of these channel genes in a tissue-specific manner.

*Formerly LNVS - transferred on August 1, 1992

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02874-01 LNC

PERIOD COVERED

February 7, 1993 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Studies of $GABA_A$ Receptor Expression During CNS Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Lawrence C Mahan, Ph D.	Research Cell Biologist	LNC, NINDS
Others:	Peng-Xin Lin, M.D	Visiting Associate	LNC, NINDS
	Peter M. Geiger	Biologist	LNC, NINDS

COOPERATING UNITS (if any)

A. Thierry, LTCB, NCI; M. Eiden, LCB, NIMH

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

Molecular Neurosciences

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.1	PROFESSIONAL: 1.1	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects [ ] (b) Human tissues [x] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have employed *in situ* hybridization histochemistry (ISHH) studies to investigate the embryonic and early postnatal expression of subunit mRNAs of the $GABA_A$ receptor in the developing rat CNS. The results demonstrate the early (E14-E15) and restricted expression of specific ($\alpha_{2/3/5} > \alpha_{1/4} >>> \alpha_6$; $\beta_3 > \beta_2 >> \beta_1$; $\gamma_{2S} >> \gamma_1/\gamma_{2L}/\gamma_3$) subunit mRNAs in certain brain regions concurrent with or shortly after neurogenesis. Little data exist as to the nature of the developmental cues, either environmental or intrinsically programed, that direct these patterns of expression. In addition, while there appears to be compelling evidence for a developmental role for GABA and its receptor, there is very little fundamental information about the functions, either individually or in combination, of specific subunits in embryonic neurons. We have chosen to investigate two *in vitro* systems: pluripotent stem cells, of both cell line and primary origin, that differentiate under controlled conditions into neurons and express GABA synthetic capability and subunits of the $GABA_A$ receptor. Preliminary studies on retinoic acid-induced differentiation of murine P19 embryonic carcinoma cells indicate differentiation into neurons (50%), glia (20-30%) and fibroblast-like cells within 72 hr. We have used PCR to determine the temporal expression of subunit mRNAs and initial characterizations appear to be in close agreement with ISHH studies *in vivo*. Parallel studies are to be carried out on differentiating neuroprogenitors isolated from olfactory bulb and cerebellum. These results will be correlated with electrophysiological and other functional characterizations of channel activation. *In vitro* and *in vivo* models of neuronal-glial interactions will be explored to provide insight into the developmental role of the embryonic expression of the GABAergic system. Molecular biological approaches in particular, the use of antisense phosphorothioate oligodeoxynucleotides and the expression of antisense episomal vectors, aimed at altering the expression of components of the GABAergic system in embryonic and postmitotic neurons are currently under development. To this end, we are constructing alternative expression vectors capable of sustained expression in precursor and post-differentiated neurons to effect changes in the GABAergic phenotype. It is hoped that these approaches may provide both a view and permit manipulation of the earliest expression of subunit genes of the $GABA_A$ receptor under conditions of more defined cellular interactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS -02019-21 LNP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Properties Developing on CNS Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS Others (LNP, BNP, DIR, NINDS): A.E. Schaffner, Biologist; M.K. Walton, Senior Staff Fellow; A. Y. Valeyev, Visiting Scientist; J Vautrin, Visiting Scientist; J-M. Mienville, Visiting Fellow; Q Y. Liu, Visiting Fellow; R. Serafini, Visiting Fellow; N. Hardegen, Chemist; V. Dunlap, Bio Lab. Technician, K.-M. Tang, Special Volunteer

COOPERATING UNITS (if any)

G.D. Lange (ICS, NINDS); P. Skolnick, G Wong (Laboratory of Neuroscience, NIDDK); K. Torimitsu, NTT Basic Research Labs, Tokyo, Japan

LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 7.9	PROFESSIONAL:: 6.9	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Electrophysiological and optical recording techniques are used primarily to elucidate the development, differentiation and cellular distribution of physiologically important properties expressed by vertebrate CNS neurons Electrical studies involve direct, high-fidelity amplification of ion fluxes generated either in single cells or patches or in synaptically coupled pairs of cells maintained in monolayer culture. Optical recordings include indirect measurements of membrane potential or of intracellular ion concentration in small populations (50-100) of cultured cells Principal findings this year include: 1) Na_o^+-dependent action potentials and underlying voltage-dependent Na^+ and Cl currents are expressed as early as embryonic (E) day 12 in telencephalic neurons, when most of the cells are still actively proliferating; 2) electrical and dye coupling among embryonic cells in intact telencephalon is greatest at E12 and then decreases during embryogenesis; 3) micromolar GABA activates Cl conductance with heterogenous properties in neuroepithelial cells from the spinal cord at E13; 4) GABA-activated Cl channels are not markedly sensitive to classical antagonists of GABA at Cl channels; 5) embryonic chick telencephalic cells initially exhibit depolarizing GABA receptors that decrease free cytoplasmic Cl (Cl_c) and increase free cytoplasmic Ca^{2+} (Ca_c^{2+}) but after 1 week in culture, receptor activation leads to an increase in Cl_c and decrease in Ca_c^{2+}; 6) E17 rat spinal cord cells initially express depolarizing GABA receptors that are more effective than receptors recorded in postnatal cells in terms of dose-response characteristics and desensitizing properties; 7) initially GABA is released in a continuous, tonic manner from embryonic neurons before it mediates transient signals; 8) dynamic interconversion between tonic and transient forms of release is correlated with mechanisms of intracellular Ca^{2+} homeostasis; 9) GABA included in the intracellular recording saline generates tonic activation of Cl channels in non-neuronal cells transfected with GABA receptor mRNAs; 10) GABA activates longer-lasting Cl channels in neurons dissociated from the embryonic relative to postnatal and adult thalamus; 11) shortening of GABA-activated Cl channels parallels changes in the intracellular Cl concentration; 12) the Cl channel-activating effects of GABA applied to embryonic thalamic neurons long outlast the application period and are sensitive to pressure-applied saline but not to GABA uptake blockers

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02330-16-LNP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Biological Studies of Developing CNS Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.L. Barker, Chief, LNP, BNP, DIR, NINDS Others (LNP, BNP, DIR, NINDS): A.E Schaffner, Biologist; W. Ma, Senior Staff Fellow; R. Somogyi, Senior Staff Fellow; D Maric, Visiting Fellow; I. Maric, Visiting Fellow; T.N Behar, Microbiologist; N Hardegen, Chemist; S V. Smith, Biologist; V. Dunlap, Bio. Lab. Technician; M. G Alessandri, Visiting Fellow; K -M. Tang, Special Volunteer, X. Wen, Special Volunteer

COOPERATING UNITS (if any)

L. Hudson (Lab.Molecular & Viral Pathogenesis, NINDS); L. Mahan (LCB, NIMH); J. Hickman (SAIC, Fairfax, VA); D. Stenger (Naval Research Laboratories, Washington, DC)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 10.9	PROFESSIONAL: 8.0	OTHER: 2.9

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects [ ] (a1) Minors [ ] (b) Human tissues [X] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Flow cytometry, discontinuous-gradient cell isolation, amino acid analysis, dissociated cell culture, immunoblots, cell migration, immunochemistry, in situ hybridization and PCR methods are applied to embryonic/early postnatal rat CNS tissues to study the development, differentiation and cellular distribution of transmitter, transmitter-related enzymes and their corresponding receptors. During the past several years, we have focussed intensely on GABA, which is transiently expressed in a virtually ubiquitous manner during CNS development before it becomes restricted to fast inhibitory synapses in the adult. In FY 93 we investigated the following: 1) transcripts encoding two GABA-synthesizing enzymes emerge at E13 in the thalamus and are expressed by virtually every cell during development; 2) transcripts encoding 8 GABA receptor subunit proteins emerge at E14 in the thalamus, are expressed until the second week postnatal when all but two become undetectable with these latter two being joined by two new transcripts to complete the adult GABA receptor form; 3) PCR reveals transcripts encoding 9 GABA receptor subunit proteins at E12 in the spinal cord when only one subunit can be detected by *in situ*; 4) *in situ* reveals three distinctive patterns of transcript coexpression in the cord: one exclusively in the neuroepithelial proliferative zone, one in most cells during the embryonic/postnatal period and one emerging during postnatal differentiation; 5) by densitometry of *in situ* signals and PCR of the 4 transcripts that remain in the adult, it is clear that all 4 are several-fold more abundant during development; 6) the chemokinetic effects of GABA on embryonic spinal cord cells are dose-dependent, with cells migrating to fM and pM concentrations, and age- and region-dependent, with ventral cells migrating before dorsal cells; 7) chemokinetic effects of GABA can be mimicked by both baclofen and muscimol, suggesting novel GABA receptor structure-activity relations; 8) pertussis toxin inhibits GABA-induced chemokinesis, but not chemotaxis induced by NGF, implicating G protein-mediated signal transduction in these novel effects of GABA's; 9) aM levels of GABA, muscimol and baclofen all release cytoplasmic free Ca^{2+} during the embryonic period of cortical development; 10) embryonic cells differentiating in culture can be organized into specific patterns using defined surfaces and substrates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-01659-25 LNP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synaptic Contact of Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: A Lasansky, Unit Chief, LNP, BNP, DIR, NINDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The chloride-dependent ON-OFF response to illumination recorded with patch electrodes from depolarizing retinal bipolar cells following run-down of the direct photoreceptor input was blocked by 1 mM kynurenic acid or a mixture of 20 µM CNQX and AP-7, presumably because these glutamate antagonists suppressed the responses of third-order neurons. Since CNQX alone only blocked about 80% of the chloride-dependent input, it may be assumed that the responses of the presynaptic third-order neurons are mediated by a combination of kainate and NMDA receptors. When run-down of the direct photoreceptor input was prevented by using high-resistance electrodes, the mixture of CNQX and AP-7 increased the amplitude of the inward current elicited by illumination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02631-10 LNP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structure and Function in Retinal Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Ralph Nelson	Unit Chief, LNP, NINDS
Others:	Michael A. Freed	Staff Fellow, LNP, NINDS

COOPERATING UNITS (if any)

Department of Physiology, University of Vienna, Austria (Renate Pflug)
Department of Physiology, University of Utah School of Medicine, Salt Lake City (Helga Kolb)
Department of Psychology, Queens College, City University of New York (Thomas Frumkes)
Department of Anatomy, University of Pennsylvania, Philadelphia (Peter Sterling, Robert G. Smith)

LAB/BRANCH

Laboratory of Neurophysiology, BNP, DIR, NINDS

SECTION

Neural Circuitry Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neural organization and neural interactions in mammalian retinas are investigated using intracellular electrophysiology, electron microscopy, and pharmacology

A hyperpolarizing amacrine cell, identified as type A8, has been penetrated with a sharp microelectrode, studied electrophysiologically, injected with HRP, and observed in the electron microscope. Cone dominated physiology, and predominant connections with hyperpolarizing cone bipolar cells in the inner plexiform layer were observed

Suppressive rod-cone interaction (SRCI) is a lateral interaction whereby dark-adapted rods antagonize cone signals. GABAergic effects on SRCI have been investigated in horizontal and ganglion cells in cat retina. Bicuculline and picrotoxin had no effects on SRCI in horizontal cells, but appeared to block the effect at the ganglion cell level by increasing dark-adapted, but not light-adapted, cone-signal amplitudes. Thus, SRCI may originate at multiple sites in the retina with different sensitivities to $GABA_A$ antagonists

In lower vertebrates, dopamine reduces receptive field size of retinal horizontal cells through metabotropic uncoupling of inter-horizontal-cell gap junctions. The effects on mammalian retina were studied. Horizontal cell receptive fields in cat and rabbit retinas were little influenced by dopaminergic ligands (dopamine, apomorphine, SKF38393, SCH23390, sulpiride) in the 70-700 µM range. Small observed changes ($\pm$ 20% in space constant) appear near the limit of measurable repeatability. Results suggest that dopamine is not a major modulator of receptive fields in mammalian horizontal cells.

Voltage noise in cat ON-beta ganglion cells increases during photic stimulation. Such noise may provide insight into the nature of synaptic transmission between bipolar and ganglion cells. Two types of events were identified: large (~1 mV) monophasic depolarizing signals which may reflect action potentials leaked through gap junctions from adjacent ON-beta cells, and a smaller Gaussian distributed noise. Analysis of the latter suggests a quantal size of about 12 µV and a release rate of about 100 quanta per second per bipolar-to-beta-cell synapse

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-NS-02767-06 LNP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Image Processing and Analysis of Cellular Structures

PRINCIPAL INVESTIGATOR (List other professional persons below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T G Smith, Jr. Unit Chief, LNP, BNP, DIR, NINDS
Others (LNP, BNP, DIR, NINDS): Anne E Schaffner, Biologist, T N Behar, Technician

COOPERATING UNITS (if any)

G D Lange, W H Sheriff, Jr (IACS, NINDS), W B Marks (LNLC, NINDS); E A Neale, L M Bowers (LDB, NICHD); Seth R Goldstein, (BEIP, NCRR); Andreas Reichenbach, Kurt Brauer (Leipzig University, Germany); Robert Porter (Monash University, Australia); R D Mckinnon, M Dubois-Dalcq (LVMP, NINDS)

LAB BRANCH

Laboratory of Neurophysiology

SECTION

Unit on Sensory Physiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
1.2	1.1	1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews
☐ (b) Human tissues
☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to employ the concepts of Mandelbrot's fractal geometry to the quantitative studies of central nervous system neurons, and other cell types grown in tissue culture or from whole animals. We do this by employing image processing techniques to measure the fractal dimension (D), which is a measure of the complexity of the structure under investigation. In particular, the D relates to the degree of branching (e.g., of dendrites), the ruggedness of borders and the degree of space-filling of the object of interest.

We have undertaken, in separate studies, how the fractal dimension changes during the differentiation and growth of glial cells from different sources (optic nerve and brain) and of neurons in tissue culture. We have found that both optic nerve and brain-derived oligodendrocytes differentiate faster and to a greater extent than do both types of astrocytes, and that nerve derived glia also differentiate faster and to a greater extent than do brain-derived glia. Interestingly, the rates of differentiation, as measured by D, can be described by a single time constant. The work on cultured spinal neurons shows that the cells can be classified into four groups on the basis of the number of their primary dendrites and that they differentiate in a similarly simple fashion, with each of the four groups having distinctive final D values and time constants. We have proposed that D is a useful, quantitative and unbiased measure of morphological differentiation.

We examined the Ds of cerebellar Purkinje cells from nine vertebrate species, ranging from birds through marsupials to mammals, including man. This indicates a phylogenetic constancy of Purkinje cell morphological complexity going back at least as far as birds in the evolutionary tree.

We have begun studies of the development of the internal and surface structures of cultured rat hippocampal neurons with fluorescence and confocal microscopy in order to localize the position of GABA and glutamate boutons. We find that GABA boutons are located almost exclusively on somata and proximal dendrites, while glutamate boutons are mainly on peripheral dendrites but occasionally on proximal dendrites and less so on somata.

We continue in our efforts to improve the performance of our confocal microscope with no moving parts by changes in design and components.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01NS02034-21 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Oligodendrocyte Lineage of Rodent and Man

PRINCIPAL INVESTIGATOR (List other authors below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	M Dubois-Dalcq, M D	Chief, LVMP	LVMP, NINDS
Others	R Vosskuhl, M D	Comm Officer	LVMP, NINDS
	U Tontsch, Ph D	M S Fellow	LVMP, NINDS
	L Millward, Ph D	IRTA	LVMP, NINDS
	R Rusten	Biol Lab Techn	LVMP, NINDS
	N Gogate, Ph D	Special Volunteer	LVMP, NIND
	L Verma, B S	Special Volunteer	LVMP, NINDS

COOPERATING UNITS (if any)

Dr I Duncan and D Archer, Univ of Wisconsin, Dr C Kufta, Neurosurgery Branch, NINDS, and Dr M O'Connor, University of Pennsylvania, Philadelphia, PA

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Neural and Molecular Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
5.0	3.3	1.7

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Myelin-forming cells enwrap axons to allow fast conduction along major nerve tracts. In multiple sclerosis (MS) and some CNS viral diseases, damage to myelin-forming cells result in important neurological dysfunction. Our studies on developing rat oligodendrocytes have shown that platelet-derived and basic fibroblast growth factors (PDGF and bFGF) trigger migration and mitosis of oligodendrocyte progenitors (OP). During differentiation oligodendrocytes express transforming growth factor (TGF)-beta 1, 2 and 3 isoforms and secrete an inhibitor of cell mitosis that can be neutralized with antibodies to TGF beta. Thus, TGF-beta produced by differentiating cells may limit growth and promote oligodendrocyte differentiation in an autocrine manner. To further our understanding of oligodendrocyte differentiation signals, we characterize the myelinating properties of a rat OP cell line. These cells were "tagged" with the Lac Z gene and grafted into the spinal cord of dysmyelinated rats where they myelinated up to 7 mm of the dorsal tracts. Another gene tag (MX) has been used to follow the migration of grafted cells which appear to be attracted toward a demyelinating lesion in mice.

Our studies of the human oligodendrocyte lineage demonstrate that the human myelinated brain contains a discrete subpopulation of glial cells expressing two oligodendrocyte-specific and developmentally regulated genes, the PDGF receptor alpha and the Myelin Transcription Factor 1 (MyT1). In addition, a substantial proportion of glial cells of the adult human white matter express the early forms of myelin basic protein (MBP) transcripts which are characteristic of the premyelinating stage. These genes are also expressed in cultured human oligodendrocytes and/or their precursor cells. Contrary to what happens with adult rat OP, bFGF and PDGF fail to stimulate human OP to divide. However, bFGF induces human oligodendrocytes to rapidly regenerate their processes *in vitro* and to dedifferentiate into OP expressing MyT1 and early MBP transcripts. Thus, phenotypic plasticity rather than mitogenic potential may account for the regeneration of myelin-forming cells in the adult human brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02528-12 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Developmental Control of Gene Expression in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L D Hudson, Ph D	Section Chief	LVMP, NINDS
Others:	J G Kim, Ph D	Sr Staff Fellow	LVMP, NINDS
	C Wiese, Ph.D	Research Volunteer	LVMP, NINDS
	J Wrathall, Ph D	Research Volunteer	LVMP, NINDS
	M Ranjan, Ph D	IRTA	LVMP, NINDS
	A Warrington, Ph D	Research Volunteer	LVMP, NINDS
	J Berndt, B S	Microbiologist	LVMP, NINDS
	N Ko, B S	HHMI Student	LVMP, NINDS

COOPERATING UNITS (if any)

H deF Webster, Lab. of Experimental Pathology, NINDS; J Barker, Lab of Neurophysiology, NINDS; A Gronenborn, Lab of Chemical Physics, NIDDK; R Armstrong Dept of Anatomy, USUHS, T Bray-Ward, Dept of Genetics, Yale University

LAB BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section of Molecular Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 5 3	PROFESSIONAL 3 6	OTHER 1 7

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanisms that dictate the final program of gene expression in a fully differentiated cell can be revealed by starting at either end of the regulatory cascade To examine the series of controls operating on cells of the oligodendrocyte lineage, we have begun with one of the final targets of regulation in myelinating glial cells, proteolipid protein (PLP) Expression libraries were screened with DNA probes corresponding to PLP cis -regulatory elements by a method that relies on the detection of DNA-protein interactions five novel clones (named MyTI-V for Myelin Transcription Factor) were isolated The most extensively characterized clone of this screen, MyTI, is highly expressed in the developing nervous system, primarily in the nuclei of progenitor cells When progenitors are induced to differentiate into oligodendrocytes *in vitro*, the nuclear form of MyTI disappears and the cells transiently express MyTI in the cytoplasm The consensus binding site for MyTI is represented in the PLP promoter as well as in other myelin gene promoters, presenting a mode of coordinate control for these genes during oligodendrocyte development The isolation of clones encoding transcriptional regulatory proteins permits a search for the growth factors and other molecules that are critical to the initiation and maintenance of myelin gene transcription during development and regeneration In a spinal cord contusion model, we have found that the putative myelin transcription factor, MyTI, is dramatically up-regulated following injury The expression of MyTI precedes the induction of myelin expression and therefore provides a handle for examining the molecular events underlying the remyelinating state Mutations in the major myelin protein PLP result in a devastating loss of white matter in the X- linked disease of man (Pelizaeus-Merzbacher disease) and animals To investigate whether the PLP locus has multiple roles in myelinating cells that would explain the pleiotropic phenotypes observed in the PLP mutants, we generated transgenic mice which express either PLP or its alternatively spliced isoform, DM20 Neither the PLP transgene nor the DM20 transgene alone restored myelin expression in mice Only a combination of the two transgenes substantially increased myelination, suggesting that the two alternatively spliced products of the PLP locus perform distinct functions in oligodendrocytes Thus, the transgenic approach offers a suitable *in vivo* system for dissecting gene function, and will continue to be applied to our studies of other genes in the nervous system

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1NS02852-02 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuro and Gliogenesis in the Developing Human Brain and Uses for Transplantation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Carlo S Tornatore, M D	Senior Staff Fellow	LVMP, NINDS
Others	Walter Atwood, Ph D	Staff Fellow	LVMP, NINDS
	Blanche Curfman, B S	Microbiologist	LVMP, NINDS
	Eugene O Major, Ph.D	Section Chief	LVMP, NINDS
	Renee G Traub, B S	Microbiologist	LVMP, NINDS
	Karen Meyers	Biologist	LVMP, NINDS

COOPERATING UNITS (if any)

Surgical Neurology Branch, NINDS, Department of Neurologic Diseases, Brigham and Women's Hospital, Harvard Medical School

LAB BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Molecular Virology and Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAF YEARS	PROFESSIONAL:	OTHER:
2 0	1 5	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The developing human central nervous system (CNS) consists of pleuripotent cells which mature into astrocytes, oligodendrocytes and neurons We have begun examining fetal brain from different gestational ages to determine at which gestational age differentiated cells can be identified The constituent elements of the developing CNS can be separated from one another by a mechanical method allowing study of individual cellular components This also allows the production of highly purified cultures of fetal neurons or astrocytes which can be used in cell culture models of HIV-1 or other neurotrophic infections These brain cell cultures can also be useful in testing transplantation protocols for therapy of neurodegenerative disorders We have previously developed an immortalized fetal astrocyte line (SVG) and have implanted them into the basal ganglia of six rhesus monkeys In the rhesus CNS, the SVG cells survived without rejection or induction of a graft versus host response Furthermore, no tumor formation or changes from normal behavior were noted These data demonstrate the survivability of an astrocyte cell line as a xenograft, and suggest that these cells could act as a drug delivery system if genetically modified To this end, we have modified the SVG cells by insertion of a tyrosine hydroxylase gene construct These cells, SVG-TH, could potentially serve as an alternative to neural grafts of primary tissue in transplantation studies

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1NS02790-05 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Insertional Mutations in Transgenic Mice

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H Arnheiter, M.D	Visiting Scientist	LVMP, NINDS
Others:	C Hodgkinson, Ph D	Visiting Fellow	LVMP, NINDS
	A Nakayama, M D , Ph D	Visiting Fellow	LVMP, NINDS
	S Skuntz, B S	Biologist	LVMP, NINDS
	E Meier, Ph D	Sr Staff Fellow	LVMP, NINDS

COOPERATING UNITS (if any)

Nancy Jenkins, Ph D , Neal Copeland, Ph.D , Karin Moore, Ph D., Eirikur Steingrimsson, Ph.D., ABL-Basic Research Program, NCI-CRDC, Frederick; M Tachibana, M.D , LMO, NIDCD

LAB BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
3 9	2 9	1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mice homozygous for mutations at the *microphthalmia* (*mi*) locus have varying degrees of melanocyte deficiencies in skin, eye and ear, and varying deficiencies in mast cells, NK cells, and osteoclasts. Depending on the mutant allele, such mice are white, microphthalmic, and hearing-impaired. Heterozygotes either have no visible phenotype, or a mild melanocyte deficiency. Heterozygous combinations of certain *mi* alleles show interallelic-interactions, some aggravating and some lessening the severity of the phenotypes seen in corresponding homozygotes. Using a transgenic insertional mutation at the *mi* locus, we have isolated a gene whose expression is disrupted in the transgenic mice. This gene encodes a novel member of the basic-helix-loop-helix-zipper (bHLH-Zip) family of DNA-binding transcription factors, and is expressed in wild type mice in the melanocytes of the retina, ear and skin, and in mast cells. The gene is mutated in six different, independent *mi* alleles, suggesting that it is indeed the only one responsible for the pleiotropic mutant phenotype. Members of this class of genes have wide ranging roles in gene regulation, cell proliferation and development in species as divergent as yeast and humans. *In vitro*, bHLH-Zip proteins act as homodimers and heterodimers, a fact that provides a rationale for the phenomenon of interallelic interactions and suggests that dimerization of these factors also operates *in vivo*. Mutations at *mi* have been proposed as models for certain forms of human Waardenburg syndrome and for human vitiligo. The recent isolation of the human Mi cDNA will enable us to study potential mutations in these diseases.

A second insertional mutation we have chosen to analyze is characterized by vertebral abnormalities similar to those seen in mutations in the *pax 1* transcription factor gene on chromosome 2. This insertion, however, is not allelic with *pax 1*, which suggests that the gene interrupted by insertion may represent a target gene of *pax-1*. Our analysis has proceeded to the isolation of a region flanking the insertion and the characterization of an associated genomic deletion. An mRNA derived from this locus is currently being analyzed. The mutation is reminiscent of certain human vertebral diseases, and the molecular analysis of the mouse gene responsible for the phenotype may lead to the isolation of the corresponding human gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1NS02698-08 LVMP

RIOD COVERED

ctober 1, 1992 through September 30, 1993

TLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

ology of Mammalian Homeodomain Proteins

RINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

I: H Arnheiter, M.D Visiting Scientist LVMP, NINDS

OOPERATING UNITS (if any)

V J Mitchell, D V M , Ph.D , Sr. Staff Fellow, LNEP, NINDS, W F. Odenwald, Ph.D , Staff Fellow, LNC, INDS; Shang-Ding Zhang, Ph D , Visiting Fellow, LNC, NINDS

AB BRANCH

aboratory of Viral and Molecular Pathogenesis

ECTION

iral Pathogenesis Section

NSTITUTE AND LOCATION

INDS, NIH, Bethesda, Maryland 20892

OTAL STAFFYEARS. 0	PROFESSIONAL:	OTHER:

HECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

UMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

his project has been terminated as part of the LVMP activity (will continue as part of LENP)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS007423-07 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on the space allocated to publication.)

Mechanisms of Viral Pathogenesis

PRINCIPAL INVESTIGATOR (List other authors below the Principal Investigator.) Name, title, laboratory, and institute affiliation

P	E Meier, PhD	Sr Staff Fellow	LVMP, NIAIDS
Others	H Arnheiter, MD	Visiting Scientist	LVMP, NIAIDS
	M Obberghansli, PhD	Visiting Fellow	LVMP, NIAIDS
	H Lee Heilman, PhD	CRTA Fellow	LVMP, NIAIDS

COOPERATING UNITS (if any)

R Ramabadran, PhD Laboratory of Neurochemistry, NINDS

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Viral Pathogenesis Section

INSTITUTE AND LOCATION

NIAIDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
2.7	2.7	0

CHECK APPROPRIATE BOX(ES)

___ (a) Human subjects
___ (a1) Minors
___ (a2) Interviews

___ (b) Human tissues

[X] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mx proteins, dynamin, VPS1, SPO15, and MGM1 comprise a new family of large GTPases (Mr = 70,000 - 100,000) which are structurally conserved but have diverse biological activities. The alpha/beta interferon induced Mx proteins of vertebrates were originally identified as proteins that confer resistance to specific viruses. However, the antiviral activity may not be the primary function but a by-product of an as yet unknown cellular activity. The Drosophila dynamin plays a role in endocytosis whereas the function of vertebrate dynamins is unknown. The yeast VPS1 SPO15 protein is required for exocytosis and sporulation, respectively, and the yeast MGM1 protein for maintenance of mitochondrial DNA. Our studies focus on functional aspects of Mx proteins and dynamin.

Current work with Mx proteins concerns the structure function relationship. The cytoplasmic rat Mx2 and Mx3 proteins differ in several functional aspects although they are identical in all but 8 amino acids. Mx2 protein has potent anti-VSV activity, it gives granular immunofluorescent staining, and it binds to microtubules in a GTP-dependent fashion. Mx3 protein has no measurable antiviral activity, it gives diffuse staining, and does not bind to microtubules in a GTP-dependent fashion. By taking advantage of the very similar primary structures of Mx2 and Mx3 proteins we have identified in chimeric Mx2/Mx3 proteins a region close to the carboxy-terminus that is important for the antiviral activity and speckled appearance of Mx2. Interestingly, although the carboxy-terminal half of Mx2 alone was not sufficient for antiviral activity, it gave speckled staining. We are currently testing which region in Mx2 is involved in the GTP-dependent microtubule-binding activity. A different functionally important domain was identified by virtue of its ability to react with a monoclonal antibody that neutralizes the influenza virus resistance of interferon-treated mouse and rat cells and the GTPase activity of purified rat Mx2 and Mx3 proteins. The identification of functionally important domains in Mx proteins may help us to elucidate the role Mx proteins play in interferon-treated cells and the mechanism by which they inhibit viruses.

To genetically define the role dynamin plays in vertebrates we have initiated a project to knockout the mouse dynamin gene by homologous recombination in ES cells. We have isolated genomic DNA clones containing the 5' terminal part of the dynamin protein and are presently constructing a

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS01983 22 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Pathogenesis of JC Virus and Progressive Multifocal Leukoencephalopathy

PRINCIPAL INVESTIGATOR (If other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Eugene O. Major, Ph.D.	Section Chief	LVMP, NINDS
Others.	Walter Atwood, Ph.D.	Staff Fellow	LVMP, NINDS
	Katherine Conant, M.D.	Sr. Staff Fellow	LVMP, NINDS
	Blanche Curfman, B.S.	Microbiologist	LVMP, NINDS
	Linda Durham, M.S.	Biologist	LVMP, NINDS
	Carlo S. Tornatore, M.D.	Sr. Staff Fellow	LVMP, NINDS
	Renee G. Traub, B.S.	Microbiologist	LVMP, NINDS
	Kei Amemiya, Ph.D.	CRADA	Igen, Inc.

COOPERATING UNITS (if any)

Depart. of Neurology, Univ. of Miami, Depart. of Neurology, VA Hospital, Washington, Dc, Medical Neurology Branch, NINDS. Animal Health Care Section NINDS. AIDS Clinical Trial ORNP, OAR, OD, NIH

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Molecular Virology and Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD

TOTAL STAFF-YEARS	PROFESSIONAL	OTHER
3.5	2.0	1.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigations of JCV induced progressive multifocal leukoencephalopathy (PML) are being carried out on clinical specimens, in tissue culture, and biochemical analysis of host cells. JCV DNA was detected in peripheral lymphocytes in more than 90% of PML patients using PCR analysis. Many of these patients had AIDS as the underlying immune disorder. In HIV-1 seropositive individuals without PML, more than 50% of the individuals were found to have JCV in their peripheral lymphocytes. This later group would be at risk to develop PML in the future. JCV DNA was found in bone marrow and kidney tissue three years prior to the onset of PML in a patient with Wiskott/Aldrich syndrome, and latter found in bone marrow and brain samples taken at the time of autopsy. This latter case suggests that JCV can be latent in cells in bone marrow, and in addition, with the finding of JCV in peripheral lymphocytes, suggest that JCV could be spread to the CNS by a hematogenous route. Expression vectors under the control of the prototype Mad-1 or 'brain' type strain Mad-8 regulatory region are being constructed to examine what tissue and cell type can influence JCV gene expression. Both chloramphenicol acetyltransferase and β-galactosidase expression vectors are used in transfection studies to answer the question of tissue specific and cell specific expression of JCV. Biochemical analysis of nuclear proteins from human fetal brain and human B cells were studied to determine if similar proteins were involved in JCV gene expression in these tissues. Nuclear proteins from both these human cell lines were able to specifically interact with identical nucleotide sequences in the JCV regulatory region. One of these protein factors was identified as a nuclear factor 1 (NF-1) protein and the other a c-Jun-like factor. Within the regulatory region of JCV, there were several NF-1 protein binding sites. The c-Jun binding sites were either adjacent or overlapped all the NF-1 binding sites located in the regulatory region. A similar association of putative NF-1 and activator protein binding sites was found in many other genes expressed in the brain. These results suggest that human brain cells and B cells may contain similar factors which can regulate the expression of JCV in these tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS02830-03 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of HIV Transcription In Vitro and In Vivo

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	E Verdin, M D	Senior Staff Fellow	LVMP,NINDS
Others	A ElKharroubi, Ph D	Visiting Fellow	LVMP,NINDS
	C Van Lint	Special Volunteer	LVMP,NINDS
	P Paras, B S	Biologist	LVMP,NINDS
	M Jonn, B S	HHMI/NIH Scholar	LVMP,NINDS

COOPERATING UNITS (if any)

Arsene Burny, Ph D , University of Brussels, Brussels, Belgium.

LAB BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Neural and Molecular Biology

INSTITUTE AND LOCATION

NINDS,, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS: 3 7	PROFESSIONAL: 1.2	OTHER: 2 5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The objective of this project is to define the molecular mechanisms controlling the expression of HIV-1 transcriptional regulation *in vivo*. Because of the emerging role of chromatin in modulating transcriptional regulatory mechanisms, we have analyzed the chromatin organization of the promoter of HIV-1 integrated in chronically infected cell lines. We have found that 2 regions, localized in the promoter/enhancer (U3 region) and immediately downstream of the 5'LTR, respectively, are nucleosome free. The DNA separating these two domains is incorporated into a nucleosome (called nuc-1) in basal conditions, when no viral expression is noted. Following TPA or TNF-alpha treatment, two agents known to induce viral expression at the transcriptional level in our cell lines, this nucleosome is displaced or disrupted. Our efforts in this last year have been directed toward understanding the mechanism of disruption of this nucleosome following activation of viral expression. Since most chromatin remodelling takes place during DNA replication, we have examined the time course of disruption of nuc-1 following TPA or TNF-alpha treatment and found it to be essentially completed in 20 min, which is inconsistent with a requirement for DNA replication. Since this nucleosome is on the path of the transcribing polymerase, we have also examined the effect of transcription on the disruption of nuc-1. Pretreatment of the cells with alpha-amanitin had no effect on the disruption of nuc-1, indicating that this phenomenon is independent of transcription. A nucleosome-free region was also noted downstream of nuc-1, possibly indicating that DNA-binding factors are present in this region *in vivo*. We have examined this region using *in vitro* and *in vivo* footprinting and have identified several binding sites for transcription factors including Sp1, AP3, AP1 and for a new factor that we have called DBF-1. We have generated mutations in each of these sites, and have reengineered them back into infectious molecular clones of HIV-1. Current studies are examining the effect of each of these mutations on viral replication in cell lines and human primary cultures (PBMCs). The significance of these studies lies in their potential relevance for HIV-1 latency and reactivation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02789-05 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurotropism of Human Retroviruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	M. Dubois Dalcq, M.D.	Chief, LVMP	LVMP,NINDS
Others:	S. Wilt, Ph.D.	IRTA Fellow	LVMP, NINDS
	K. Nagasato, M.D., Ph.D.	Special Volunteer	LVMP, NINDS
	F. Chiodi, Ph.D.	Guest Worker	LVMP, NINDS
	J.M. Zhou	Visit. Associate Techn.	LVMP, NINDS

COOPERATING UNITS (if any)

Dr. F. Chiodi, Karolinska Institute, Stockholm, Sweden; Dr. M. O'Connor, Univ. of Pennsylvania, Phil., PA; Dr. E. Verdin, LVMP, NINDS, Drs. I. Koralnik & V. Francchini, Lab. Tumor Cell Biol., NCI; and Drs. D. Griffin and S. Wesselingh, Johns Hopkins Medical School, Baltimore, MD

LAB/BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Neural and Molecular Biology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The human retroviruses HIV-1 and HTLV-1 can both infect the central nervous system (CNS) causing the AIDS psychomotor complex and tropical spastic paraparesis, respectively. To study neurotropism of these viruses, we use primary cultures derived from adult human brain. As microglial cells are the major target cell of HIV-1 within the CNS, we have examined microglial cell tropism and molecular determinants within the V3 loop of gp 120 in 13 HIV-1 variants isolated from blood and/or cerebrospinal fluid (CSF) at early or advanced stages of AIDS. The majority of HIV-1 variants isolated from blood and CSF even in the asymptomatic stage of the disease, can infect microglial cells, although their V3 loop may differ substantially from each other. Thus, HIV-1 variants in blood or CSF of seropositive patients may infect microglia early in infection, establishing a virus reservoir in the CNS. In contrast, highly cytopathic syncytium inducing viruses, isolated at an advanced stage of the disease, are less likely to replicate in primary human brain microglia.

We have also investigated the role of tumor necrosis factor (TNF) alpha in HIV-1 encephalopathy using purified microglial cultures derived from adult human brain. Such cells are activated and express TNF alpha, just as they do in the brain tissue of patients with AIDS psychomotor complex. When infected with HIV-1 in the continuous presence of TNF alpha antibody, HIV-1 expression and virus growth in microglial cells are strongly inhibited for over a week, suggesting that TNF alpha naturally produced in this *in vitro* system may enhance HIV-1 replication. Moreover, microglial cell-derived TNF alpha may be toxic for oligodendrocytes, the CNS myelin-forming cells and cause the demyelination observed in the HIV-1 leucoencephalopathy. To investigate this possibility, we have developed an *in vitro* assay in which TNF alpha induced-cell death of purified rat and/or human oligodendrocytes can be accurately measured. Thus, TNF alpha may indirectly damage some CNS cells and enhance HIV-1 expression and spread within the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1NS028S1-02 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HIV-1 Infection in Human Fetal Brain Cell Cultures and Pediatric AIDS Brain Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Eugene O Major	Section Chief	LVMP,NINDS
Others:	Kei Amemiya, Ph D	CRADA	Igen, Inc
	Walter Atwood, Ph D	Staff Fellow	LVMP, NINDS
	Katherine Conant, M D.	Sr Staff Fellow	LVMP, NINDS
	Karen Meyers	Biologist	LVMP, NINDS
	Carlo S Tornatore, M D	Sr Staff Fellow	LVMP, NINDS

COOPERATING UNITS (if any)

Pediatric Branch, NCI, NIH and Department of Pathology, National Children's Hospital, Washington, DC

LAB BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Section on Molecular Virology and Genetics

INSTITUTE AND LOCATION

National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892

TOTAL MAN YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Possible causes of HIV-1 induced neurotoxicity include infection of select populations of glial cells. We have established a useful model of HIV-1 infection in human fetal brain cell cultures to study the mechanisms by which this may occur. Through either infection with virions or transfection with proviral DNA, human fetal astrocytes quickly develop a non-cytopathic but productive infection which gradually diminishes to a persistent infection without viral expression at the RNA or protein levels. However, HIV-1 expression can be reactivated by the cytokines TNF-alpha and IL-1 beta as well as by phorbol myristic acid (PMA), a potent activator of protein kinase C. PKC inhibitors such as H-7, an isoquinolone, can block reactivation by TNF-alpha and PMA, an effect which in the case of PMA is likely due to a reduction in the transcriptional activator NF kappa-B. Intracellular pathways involved in HIV-1 reactivation appear to be cell-type dependent as other factors known to induce HIV-1 from human monocyte cells such as GM-CSF, IL-6, IL-2 and interferon do not activate HIV-1 from astrocytes. Extraction of mRNA following stimulation with TNF-alpha or IL-1 beta demonstrates the presence of mRNA for nef, tat and rev proteins, of which nef is the most abundant and longest lasting. We have evidence that infection of glial cells is important *in vivo* in that tissue from 4 of 12 pediatric AIDS brains has revealed glial fibrillary acidic protein (GFAP) positive astrocytes with positive hybridization to HIV-1 radiolabeled probes. In several of these sections, there was no evidence for the HIV-1 antigens p24 and gp41. These results suggest that astrocytes may harbor an undetectable HIV-1 proviral DNA that can be activated in the brain through cytokines. TNF-alpha and IL-1 beta are reported to be present in AIDS brain tissue in high concentrations. Other work has shown that these cytokines are produced by astrocytes in response to HIV infection. Further study of the molecular and biochemical aspects of HIV-1 infection of astrocytes and its clinical correlates in pediatric AIDS encephalopathy are currently in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS02818-04 LVMP

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pseudotypic Defective Interfering HIV Particles as an Antiviral Therapy for AIDS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	M Schubert, Ph D	Section Chief	LVMP,NINDS
Others.	A C Banerjea, Ph.D	Sr. Staff Fellow	LVMP, NINDS
	C -J Chen, Ph D	Visiting Associate	LVMP, NINDS
	S -Y Paik, Ph D , Ph D	Visiting Fellow	LVMP, NINDS
	G G Harmison II, M S	Chemist	LVMP, NINDS
	B Lewis, B S	Biol Lab Techn	LVMP, NINDS

COOPERATING UNITS (if any)
A Perelson and G Nelson, Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, New Mexico

LAB BRANCH
Laboratory of Viral and Molecular Pathogenesis

SECTION
Viral Replication Section

INSTITUTE AND LOCATION
NINDS, , NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
5 3	4 4	0 9

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Extensive efforts in the development of drugs or therapeutic vaccines against HIV-1 have not been successful Gene therapies, which involve the intracellular immunization of HIV-1 susceptible cells , face major challenges because of the diversity of HIV-1- susceptible cells The goal of this study is to develop an antiviral strategy against HIV-1 based on a defective interfering HIV-1 particle which interferes with the replication of wild type virus If all elements of this strategy perform as hoped, an equilibrium of wild type and defective HIV-1 may be achieved which could potentially stabilize both virus load and T4 cell count and thereby delay the onset of AIDS

Several candidate defective interfering HIV-1 vector constructs (HD DNAs) were further evaluated:

1 The synthesis of Nef protein encoded by some of the HD DNAs was verified by immunofluorescent staining Expression of the chimeric CD4/Env protein from two constructs which contain additional gag gene inserts was drastically reduced, suggesting that they may not be useful for the strategy

2 HIV-1 released after cotransfection with HD DNAs appeared to be less infectious, suggesting a difference in the composition of released virus

3 Several weeks of cocultivation of cells transfected with HIV-1 and HD DNA showed the presence of polyadenylated HD RNA in cell supernatants and the presence of excess HIV-1 RNA

4 A recipient cell line for HD RNAs was selected Cocultivation with HIV-1 and HD particle producing cells did not show a transfer of HD RNA at low sensitivity of the assay

5 Packaging of an HIV-1 helper virus RNA into virus particles was detected despite the fact that the RNA lacks the "essential" cis-acting HIV-1 packaging signal

6 Electron microscopy of HD virus and HIV-1 budding from cells showed expected morphological differences in the makeup of the two envelopes The insertion of the CD4/Env protein into the envelope has not been confirmed until now

7 Computer modeling of the potential use of HD viruses as antivirals seem to indicate that if all elements of the defective virus were functional, these particles may be effective in lymph nodes, the reservoir of the virus, where there is a higher density of persistently infected cells

The potential future use of HD vectors as antivirals will depend on the demonstration of HD RNA transfer to HIV-1 infected cells and the efficiency of packaging of HD RNAs relative to HIV-1 RNA

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1NS02791 05 LVMP

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Replication and Pathogenesis of Enveloped Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	M Schubert, Ph D	Section Chief	LVMP, NINDS
Others	S Y Paik, Ph D, Ph D	Visiting Fellow	LVMP, NINDS
	A C Banerjea, Ph D	Sr Staff Fellow	LVMP, NINDS
	B Lewis, B S	Biol Lab Techn	LVMP, NINDS

COOPERATING UNITS (if any)

C Y Kang, Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada

LAB BRANCH

Laboratory of Viral and Molecular Pathogenesis

SECTION

Viral Replication Section

INSTITUTE AND LOCATION

NINDS, , NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
0 7	0 6	0 1

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was initiated to study several aspects necessary for the design of viral expression vectors which can be targeted to specific cells for gene delivery. Understanding the mechanisms of viral assembly are crucial toward this goal. It is also essential to understand how the envelope of the virus, whether it is a retrovirus or another envelope virus, could be altered so it could show a specific cell tropism, while maintaining membrane fusion activity. In viral assembly, the viral matrix protein M plays a central role. It brings the genome in contact with the plasma membrane at the site of virus budding.

During the past fiscal year, we focused on the role of the matrix protein M of vesicular stomatitis virus (VSV), which is essential for viral assembly and, in part, for viral pathogenesis. Coexpression of the matrix protein with HIV-1 led to inhibition of HIV-1 replication, as indicated by a severe decrease in the level of p24 released and in the number of syncytia. We and others had earlier reported that the M protein can indiscriminately inhibit the expression of genes driven by several different RNA polymerase II promoters. If the cytopathic effect caused by M protein could be made inducible upon infection, for example, by the HIV-1 Tat protein, protection of the cell population may result. The infected cell itself may also be killed, thereby simultaneously clearing the virus from the cell population.

To test for this possibility, a temperature sensitive M protein was cloned under control of the HIV-1 LTR, and individual cell clones harboring the construct were selected and challenged by transfection with HIV-1 DNA. HIV-1 replication, as evidenced by p24 antigen release and syncytia formation, was severely decreased at the permissive temperature of M (32°C) as compared to the parental cell line which continued to support HIV-1 replication. In contrast, at the nonpermissive temperature for the M protein (40°C), the cell clones efficiently supported HIV-1 replication, like the parental cells. The fact, that the cell clones were viable at these temperatures demonstrated that basal levels of the temperature sensitive M expression from the HIV-1 LTR were not toxic to the cell. The expression of M protein at permissive temperature protected the cell population from virus spread. For the M protein to be potentially useful in a gene therapeutic approach, a lack of toxicity at the level of the uninduced wild type M protein expression needs to be demonstrated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02652-09 BFSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Collaboration and Consultation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS,
Others:	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BFSB, DIR, NINDS
	Paul S. Albert,Ph.D.	Mathematical Statistician	BFSB, DIR, NINDS
	Dallas Anderson, Ph.D.	Mathematical Statistician	BFSB, DIR, NINDS
	Gregory Campbell,Ph.D.	Chief, Analytical Biometrics Section	BFSB, DIR, NINDS
	Sherrie E. Emoto,Ph.D.	Mathematical Statistician	BFSB, DIR, NINDS
	Lisa McShane, Ph.D.	Mathematical Statistician	BFSB, DIR, NINDS

COOPERATING UNITS (if any)

Bombay Hospital, India (Dr. N. Bharucha); Peking Union Medical College, PRC (Dr. Z. Zhang); NIMH (Dr. Norman Rosenthal); Univ. of Chile, Santigao, Chile (Dr. V. Diaz); Institute for Stroke Research and Prevention, Austria (Dr. M. Brainin), Harvard Univ. Boston, MA (Dr. Q. Registein); Pharmacy, Clinical Center, NIH (Dr. K. Calis)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Office of the Chief, Mathematical Statistics Section, Analytical Biometrics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.75	3.60	3.15

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project encompasses a wide scope of statistical collaboration and consultation with laboratories and branches within the Division of Intramural Research (DIR), and with other neuroscience units outside NIH. Particular consideration is given to statistical planning and design of experiments, statistical analysis of data, and statistical inference. Examples of current studies include: clinical studies of cholesterol-lowering agents in Neimann-Pick disease, clinical course and outcome of patients with Ceredase$_{TM}$ in Gaucher's disease (Developmental and Metabolic Neurology Branch); clinical trials of felbamate for the treatment of intractable complex partial seizures, measurement of the effect of time from last seizure and seizure type on metabolic change as measured by PET; study of epilepsy progression to general tonic-clonic seizures (Epilepsy Research Branch); clinical trial of amantadine for the treatment of post-polio fatigue; optimal sampling procedures to estimate the size of a population of neuronal cells (Clinical Neuroscience Branch); examination of the relationship between MRI change and clinical status in relapsing-remitting MS, clinical trial of the effect of cyclosporine on lesion development in relapsing-remitting MS, modeling lesion recurrence in relapsing-remitting MS, clinical trial of DGS on lesion development in relapsing-remitting MS, monitoring MRI T2 weighted imaging in relapsing-remitting MS (Neuroimmunology Branch); statistical modeling of time-to-motor response complication in L-dopa- treated patients with Parkinson's disease (Experimental Therapeutics Branch); prevalence study of neurologic diseases in the Navajo tribe (Epilepsy Branch); study of abnormal facilitation response to transcranial magnetic stimuation in PD patients; identification of deficits associated with "over use" syndrome in pianists; three clinical trials of IV/IG in neuromuscular disorders (Medical Neurology Branch); evaluation of neuronal sprouting and behavioral recovery in hemiparkinsonian rats after amnion cell transplantation (Surgical Neurology Branch); validation study of consultations provided by U.S. drug information centers; case-control study of hemorrhagic stroke and alcholism in Santiago, Chile; incidence study of motor neuron disease on Guam (Neuroepidemiology Branch); development of Markov models for rapidy cycling biopolar disorder, examination of the relationship between bright light exposure and hot flashes in menopausal women (NIMH); and study of silent stroke risk factors and their implication for survival of a subsequent stroke

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02490-13 BSSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Research in Statistics

PRINCIPAL INVESTIGATOR (List other authors below senior author. Include name, organization and mailing address.)

PI:	James M. Dambrosia, Ph.D.	Chief, Mathematical Statistics Section	BSSB, DBS, NINDS
Co-PI:	Jonas H. Ellenberg, Ph.D.	Chief	BSSB, DBS, NINDS
Others:	Paul S. Albert, Ph.D.	Mathematical Statistician	BSSB, DBS, NINDS
	Dallas W. Anderson, Ph.D.	Mathematical Statistician	BSSB, DBS, NINDS
	Gregory G. Campbell, Ph.D.	Chief, Analytical Biometrics Section	BSSB, DBS, NINDS
	Sherrie E. Emoto, Ph.D.	Mathematical Statistician	BSSB, DBS, NINDS
	Lisa M. McShane, Ph.D.	Mathematical Statistician	BSSB, DBS, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.55	1.35	0.20

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [X] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project addresses statistical problems generated from collaboration with scientists in other program areas and general statistical problems of current interest. This project is a continuing activity of the Section on Mathematical Statistics and other members of the Branch. Papers have been submitted, are in review or were published in FY 1993 on the following statistical subjects: eigenvalue decompositions of data modeled by multiway arrays; validation methods for screening instruments in surveys of low prevalence disease; modeling time series for count data from a relapsing-remitting disease; modeling seasonal change in time series regression relationships; and national prevalence estimates of disease obtained by adjustment and incorporation of estimates from independent community-based surveys. Other work in progress includes: methods to improve coverage in surveys; estimation of time to event data with interval censoring; site selection for epidemiologic surveys; analysis of response surface data with spatial and temporal components; modeling of response surfaces with spatially correlated errors; application of splines to estimate model parameters of multiple correlated response surfaces; modeling effect changes of covariates in the presence of spatial correlation; combining information from negatively correlated nonlinear regressions; development of a generalized estimating equation approach for the analysis of spatially dependent binary data; application of bootstrap methods to longitudinal natural history data for the design and analysis of therapeutic trials for relapsing remitting disease; use of variance component methods to assess the precision of biochemical measurements; using Markov chain model to study three state disease processes; and sampling strategies for spatial point processes with multiple types of clustering.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02879-01

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Statistics and Neuroimaging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PPI	Gregory Campbell, Ph.D.	Chief, Analytical Biometrics Section	BFSB, DIR, NINDS
Other:	Alan Polis	Computer Systems Analyst	BFSB, DIR, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry and Field Studies Branch

SECTION
Analytical Biometrics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.55	0.75	0.80

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project undertakes the development and application of statistical methodology to neuroimaging. In particular, while brain imaging is a fundamental tool in neuroscience, the statistical treatment of the quantification of such images has lagged behind imaging technology. Numerous statistical problems have not been satisfactorily treated in the analysis of neuroimages. These include: whether and how to normalize positron emission tomography (PET) images on different groups of patients; voxel (volume element) subtraction of images to investigate regions of change in the magnetic resonance Imaging (MRI) scans of the same individuals under different tasks or drugs; multiple comparison issues to safeguard the repeatability of any inference concering a region of apparent activity (where there may be 16,000 voxels per slice); the development of techniques to exploit the spatial correlations of brain imaging as well as the temporal aspects that can occur in repeated scans over time of the same individuals; and the planning of experiments to ensure adequate power Further, the resolution of these problems is all the more crucial as the imaging technology continues to improve dramatically Research has been conducted concerning receiver operating characteristic (ROC) methodology that has direct application to the evaluation of different imaging modalities. Work in progress includes methodologies to compare imaging systems as is necessary if, for example, two different PET scanners are used in a study. Collaborative projects have begun on the analysis of data from a PET study involving familial Alzheimer's disease, and an investigation of the variability of metabolites in repeated MR spectroscopic scans (Neuroimaging Branch); and a combined PET/MRI study of induced ischemia in the motor areas of the brains of normal volunteers (Medical Neurology Branch)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02810-04 BFSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Statistical Coordinating Center for Collaborative Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Jonas H. Ellenberg, Ph.D.	Acting Chief, Collaborative Studies Section	BFSB, DIR, NINDS
Others:	Dallas W. Anderson, Ph.D	Mathematical Statistician	BFSB,DIR,NINDS
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
	Jack Panossian	Programmer	BFSB, DIR, NINDS

COOPERATING UNITS (if any)

J. William Langston, M.D. and Caroline Tanner, M.D., Neurologists, California Parkinson's Foundation; Mario Melcon, M.D., Neurologist, Regional Hospital, Junin, Argentina

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Collaborative Studies Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	0.65	0.60

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[X] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project encompasses all statistical coordinating center responsibilities for collaborative clinical studies undertaken by this Section and the Office of the Chief. A major initiative involves the study of the etiology of Parkinson's disease (PD) using the twin pair registry of the National Academy of Sciences/National Research Council. The prevalent cases of PD in the more than 6,000 twin pairs in which both members are alive, will be identified. This observational study will establish: environmental, medical and family histories of both affected and unaffected members of the twin pairs; DNA banking; and measurement of progression of disease over time. This project will investigate genetic and environmental contributions and their interactions to the etiology of PD. A Cooperative Agreement has been funded for the clinical aspects of this study. BFSB is acting as the statistical coordinating center.

A second collaborative project involves a prevalence survey of major neurologic disorders in Junin, Buenos Aires Province, Argentina. This household survey, funded by the Fundacion para la Investigacion Neuroepidemiologica, is one of the largest of its kind in Latin America. More than 20,000 residents of Junin were screened using systematic sampling techniques, and those suspected of having a disorder of interest were examined by project neurologists. BFSB has collaborated on the design and data collection phases of this study, and will collaborate on the data analysis and preparation of manuscripts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02483-13 BFSB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Predictive Value of the EEG in Febrile Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Sherrie E. Emoto, Ph.D.	Mathematical Statistician	BFSB, DIR, NINDS
Others:	Jonas H. Ellenberg	Chief	BFSB, DIR, NINDS
	Deborah G. Hertz, M.D.	Health Science Admin.	DNB, DCDN, NINDS
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS

COOPERATING UNITS (if any)

Developmental Neurology Branch, DCDN, NINDS; Neuroepidemiology Branch, DIR, NINDS; Nikola Sofijanov, M.D., Pediatric Clinic, University of Skopje, Macedonia (Yugoslavia)

LAB/BRANCH

Biometry and Field Studies Branch

SECTION

Mathematical Statistics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.35	0.25	0.10

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [X] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This population-based study will evaluate the significance of the EEG as a predictor for recurrence of seizures in those children who have had a simple febrile convulsion. Outcomes reported are febrile seizure recurrence and afebrile seizure occurrence. The evolution of the EEG pattern will be described, and patterns will be correlated with the clinical outcome. The clinical study was carried out in Skopje, Macedonia (Yugoslavia), at the Pediatric Clinic of the University of Skopje.

The study began in FY 1982. Patient accrual was completed in December, 1984, by which time approximately 400 patients with a febrile seizure, no prior complex or multiple seizures and with a normal or nonspecific abnormal EEG, were registered into the study and began the study protocol and follow-up. An additional 300 patients with a specific abnormal EEG were entered for baseline information and follow-up. Additional efforts by the clinical center were needed to collect data from those patients lacking a return visit and those who did not have long term follow-up. Final follow-up visits were completed in FY 1991. Initially 22% of the 676 children had an EEG classified as paroxysmally abnormal, which was associated by logistic regression analysis with older age, number of previous febrile seizures, preexisting motor abnormality, and focal index seizures. Statistical analysis of baseline EEG and its association with characteristics of the child and family, and the clinical characteristics of the seizure has been published. Analysis is currently being conducted to examine: the effectiveness of the initial EEG in predicting recurrent febrile seizures; the evolution of EEGs in children with febrile seizures; and the value of changes in EEGs in predicting febrile seizure recurrence. Among the more than 76% who were followed for an average of 29 months, one-fourth experienced at least one recurrent febrile seizure. The recurrence rate was 25%, 24%, and 23%, respectively, for those with normal, nonspecifically abnormal, and specifically abnormal initial EEGs. Initial EEG was also not predictive of multiple recurrences. The classification of EEG at presentation was not related to the likelihood of recurrence of febrile seizures. Additional publications from this project will be reported under ZO1 NS 02652-09 (Statistical Collaboration and Consultation). This project is complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02883-01 CNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Peripheral and Central Nervous System Peptide Neurotransmitter Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Eva Mezey, M.D., Ph.D.	Senior Investigator	CNB, NINDS/LCB, NIMH
Others:	Miklos Palkovits, M.D., Ph.D.	Visiting Scientist	LCB, NIMH
	Gyongyi Harta	Visiting Associate	CNB, NINDS
	Gabor Jakab, M.D.	Visiting Scientist	CNB, NINDS

COOPERATING UNITS (if any)

Department of Anatomy, Semmelweis University Medical School, Budapest, Hungary
First Department of Surgery, Semmelweis University Medical School, Budapest, Hungary

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Neuroanatomy, Aminergic Mechanisms Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 2.2	OTHER 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have followed up on our findings that showed the presence of certain receptors in the immune cells of the lamina propria in the gut. We have extended the studies to biopsies from human stomach and duodenum and experimental conditions in the rat. We found that the presence of the receptors studied (Histamine$_2$, gastrin and muscarinic receptors) is very similar between rats and human. We have studied several methodical problems and worked out modification of the *in situ* hybridization method so that it can be used in immune cells without the problems that made it difficult to evaluate results quantitatively. We found that there is an artificial amplification of the autoradiographic signal in cells with a large amount of oxidative enzymes (phagocytic cells) and that this can be prevented.

We have studied the distribution of a recently cloned peptide receptor in the rat CNS (GIP or gastric inhibitory polypeptide receptor). We have found that the GIP receptor has a very unique localization suggesting that it may have a role in regulating blood pressure and limbic functions. We have also mapped its peripheral distribution, and found it present in many vascular endothel cells supporting its likely involvement in the regulation of blood flow/blood pressure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02870-02 CNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Amines: Regulation and Function

PRINCIPAL INVESTIGATOR (If other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	I J Kopin, M D	Chief, CNB, Director, DIR	CNB, NINDS
Others:	S Al-Damluji, M D	Visiting Scientist	CNB, NINDS
	K Pacek, M.D	Visiting Fellow	CNB, NINDS
	Gal Yadid, Ph D	Visiting Fellow	CNB, NINDS
	J Harvey-White, B S	Technician	CNB, NINDS
	D Goldstein, M.D., Ph.D	Medical Officer	CNB, NINDS
	Joe Higgins, M D	Clinical Associate	CNB, NINDS

COOPERATING UNITS (if any)

Yigal Fraenkel, Ph D , IRTA Fellow, BMS, LBC, NIDDK

LAB BRANCH

Clinical Neuroscience Branch

SECTION

Aminergic Mechanisms

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.5	PROFESSIONAL: 2.5	OTHER 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [\] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are to: (1) examine formation, release, metabolism and disposition of brain biogenic amines and their alterations after administration of drugs or toxin-induced models of human disease; (2) determine the physiologic role of biogenic amines in mediating responses to stress, and (3) develop methods that can be adapted to study of brain biogenic amine metabolism in humans.

In vivo microdialysis has been used to monitor levels of monoamines and their metabolites in extracellular fluid in various regions of the hypothalamus and in the basal ganglia. Receptors and transporters have been examined *in vitro* using cells from different regions of brain: in cell lines cultured from the hypothalamus.

Results of studies using microdialysis have shown that norepinephrine (NE) release in the paraventricular nucleus (PVN) varies with the stressor, being greatest with immobilization and least with hypoglycemia. Furthermore, by unilateral interruption of ascending noradrenergic pathways in the brain stem, the degree of innervation of the hypothalamic nuclei can be assessed. Locally administered glycine, introduced into the regions of the tip of a microdialysis probe, elicits dose-dependent, strychnine-sensitive dopamine release. The interaction of glycine with nicotinic acetylcholine receptors in bovine adrenal medullary membranes (demonstrated *in vitro* by NMR) suggests that the amino acid modulates this receptor at one of two sites which are strychnine sensitive. Endogenous serotonin also appears to influence dopamine release from the striatum.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02213-13 CNB*

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sources and Effects of Reactive Oxygen Intermediates in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. L. Gilbert, Ph.D.	Research Physiologist	LB, NINDS

COOPERATING UNITS (if any)

NIMH, NIH, Bethesda, MD (C.C. Chiueh, N. Ozaki); Georgetown Univ. (C.A. Colton, G. Thomas, [illegible] Keri, F. Pagan), Howard Univ. (J. Stewart), Pfizer Groton, CT (R.B. Nelson)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Biophysics Section, Unit on Reactive Oxygen Species

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments have been performed on microglia, the resident macrophages in the central nervous system cultured from cerebral cortices of rat. We have previously shown that these activated cells produce the superoxide radical anion, a reactive oxygen species (ROS). ROS include also the hydroxyl radical and hydrogen peroxide. The resting concentration of intracellular calcium in the microglial cells is about 100 nM. When the calcium ionophore A23187, is added to the tissue media, superoxide radical anions are released from the microglia. The maximum intracellular calcium that can be reached by this calcium ionophore is about 300 nM. We have shown that beta A4 peptide, a part of the larger amyloid precursor protein, is aggregated into large multiple-sized fragments in the presence of activated microglia and certain other peptides.

*Formerly in LN, transferred in 1 93

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02709-08 CNB*

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Secretion of Neurotransmitters and Hormones

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Ehrenstein, Ph.D.	Research Physicist	LB, NINDS
Others:	K. Krebs, Ph.D.	Senior Staff Fellow	LB, NINDS
	M. Jia, M.D.	Visiting Associate	LB, NINDS
	M. Li, M.D.	Visiting Associate	LB, NINDS
	A. Mbuyi-Kalala, D.Sc.	Visiting Associate	LB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Biophysics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
4.8	4.8	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have previously shown that the channels through which calcium enters parathyroid cells under normal physiological conditions are voltage-independent and have single-channel conductance of about 0.6 pS. We have now determined that the steady-state influx of calcium ions through these channels is about 0.9 picoamperes per cell. The calcium pump rate required to balance this current is about 30,000 ions per square micrometer per second. This implies that the density of calcium pumps in parathyroid cells is more than twice that reported for any other cell.

We are determining the effect of fragments of parathyroid hormone (PTH) on measurements of PTH secretion. We measured the amount of intact PTH in solution by radioimmunoassay (RIA). We then added known quantities of PTH fragments, and used RIA to remeasure the amount of intact PTH. The presence of the fragments had a significant impact on the apparent quantity of intact PTH measured by RIA. Surprisingly, this quantity sometimes appeared to be reduced. A possible explanation for this effect is that some of the fragments bind to intact PTH, resulting in a reduced affinity for antibody. This could appear as a reduction in the amount of cold PTH.

*Formerly in LN, transferred in 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02717-07 CNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Catecholamine Metabolism in Health and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Graeme Eisenhofer, Ph D	Visiting Associate	CNB, DIR, NINDS
Others:	Jacques Lenders, M D	Visiting Associate	CNB, DIR, NINDS
	Douglas Hooper, B S	Chemist	CNB, DIR, NINDS
	David S. Goldstein, M D , Ph D	Medical Officer	CNB, DIR, NINDS
	Irwin J. Kopin, M D	Chief	CNB, DIR, NINDS

COOPERATING UNITS (if any)

Murray Esler, M D , Ph D , Ian Meredith, M D , Ph D , Gavin Lambert, B S , Baker Medical Research Institute, Melbourne, Australia; Peter Friberg, M D , Ph.D , Sahlgrenska Hospital, Gothenburg, Sweden

LAB BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neurochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
2.2	1.7	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The main objectives of this project are to quantify the pathways of catecholamine metabolism in the intact organism (experimental animals and humans) and establish the involvement of disturbances in these pathways in certain disease processes. Tissue, plasma or urine samples are obtained before and during pharmacological or physiological manipulations and analyzed for concentrations of endogenous and exogenous radiolabelled catecholamines and their metabolites.

Studies using a recently developed technique for the determination of plasma concentrations of the O-methylated catecholamine metabolites, normetanephrine and metanephrine, have established the relative importance of extraneuronal uptake and metabolism for the inactivation of endogenously released and circulating catecholamines. These studies are being extended to investigations of extraneuronal catecholamine metabolism and have shown that in the failing heart the contribution of this process to transmitter turnover is reduced whereas the contribution of exocytotic noradrenaline release to turnover is increased.

Other clinical studies have indicated that the lungs are a primary source of homovanillic acid, the principal metabolite of dopamine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02839-03CNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sympathoadrenal and Catecholaminergic Function in Health and Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I:	David S. Goldstein, M.D., Ph.D.	Chief, Clinical Neurochemistry Section	CNB, NINDS
Others:	Richard O. Cannon, III, M.D.	CB	DIR, NHLBI
	Anna Deka-Starosta, M.D., Ph.D.	Visiting Associate	CNB, NINDS
	Graeme Eisenhofer, Ph.D.	Visiting Associate	CNB, NINDS
	Irwin J. Kopin, M.D.	Chief	CNB, NINDS
	Karel Pacak, M.D.	Visiting Fellow	CNB, NINDS
	Arshad Quyyumi, M.D.	Senior Investigator	DIR, NHLBI
	Gal Yadid, Ph.D.	Visiting Fellow	CNB, NINDS

COOPERATING UNITS (if any)

LAB BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neurochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 2.7	OTHER: 1.3

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our laboratory develops and applies methods for assessing the function of central and peripheral catecholaminergic systems and the coordination of these systems with other homeostatic systems in health, stress, and disease. Findings this year include: (1) Position-emission tomographic (PET) scanning after systemic administration of 6-[^{18}F]fluorodopamine ([^{18}F]-6F-DA) provided a noninvasive, *in vivo* means to examine cardiac sympathetic innervation and function in humans. (2) Clinical microneurographic and tracer norepinephrine (NE) kinetic methods were applied to diagnose neurocardiologic disorders, test the "epinephrine hypothesis" of sympathetic neurotransmission, and assess catecholaminergic effects of glucocorticoids in humans. (3) *In vivo* microdialysis revealed juvenile spontaneously hypertensive rats have increased α_2-adrenoceptor-mediated restraint of catecholamine biosynthesis and NE release in the posterolateral hypothalamus. (4) Patterning of neuroendocrine responses has supported a homeostat theory of stress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02630-10 CNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical, Genetic and Biochemical Studies of Familial Alzheimer's Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

Interim P I : L E Nee, M S W Social Science Analyst OCD, CNP, NINDS

COOPERATING UNITS (if any)

Lev Goldfarb, M D , NINDS, Jordon Grafman, Ph D , NINDS; Jay Robbins, M D , NCI

LAB BRANCH

Clinical Neuroscience Branch

SECTION

Clinical Neuropharmacology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1 5	PROFESSIONAL: 0.5	OTHER: 1 0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [x] (a1) Minors
- [x] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alzheimer's disease (AD) is the most common cause of irreversible, chronic dementia. Although AD may be familial in only one-third of all cases, the main justification for studying autosomal dominant cases lies in the accuracy of diagnosis which may be inferred through postmortem examination of other affected family members. More than 270 members of 23 pedigrees with an autosomal dominant form of AD have had skin fibroblast and peripheral blood lymphoblast cultures established. These cultures serve as a renewable source of DNA and cell lines for genetic linkage, viability, and biochemical studies. Recombinant DNA technology has been applied to perform genetic linkage studies in these families with inherited AD. Approximately 75% of the amyloid precursor protein (APP) gene has been sequenced in the Canadian and Italian pedigrees. The β-amyloid peptide coding exons have been sequenced in 20 pedigrees. No mutations have been detected thus far. It appears that the Familial Alzheimer's disease locus and APP gene on chromosome 21 reside at different locations in these pedigrees.

With the departure of the PI (Dr. Polinsky) this project has been maintained mainly as a resource for future research to be developed in relation to a new Unit on Clinical Neurogenetics which has been established in the CNB. Inherited Alzheimer disease - longitudinal data collection, expansion of pedigree information and recruitment of additional family members, as well as counseling of numerous families, some followed since 1977, has continued. Collaborations have also continued although no patient was admitted during the year. In September 1993, we shall again admit families with the continued collaboration of Trey Sunderland, M D , NIMH. Longitudinal study involves LP's, PET, DNA, psychological testing, psychological and genetic counseling

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02752-06 CNB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Synthesis and Expression of Neurotrophic Agents and Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Joan P. Schwartz, Ph.D	Chief, Molecular Genetics Section	CNB, NINDS
Others:	Nobuyoshi Nishiyama, Ph.D	Visiting Associate	CNB, NINDS
	Emil Viskupic, Ph.D	Visiting Associate	CNB, NINDS
	Dahlia Minc-Golomb, Ph.D	Visiting Fellow	CNB, NINDS
	Takayuki Taniwaki, M.D.	Visiting Fellow	CNB, NINDS
	Yukihiro Sugita, M.D., Ph.D.	Special Volunteer	CNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH
Clinical Neuroscience Branch

SECTION
Molecular Genetics Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.0	6.0	1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence suggests that parallel biochemical and regulatory processes occur during normal development and following various forms of central nervous system (CNS) injury. Among these areas of particular interest are: (1) identification of CNS neurotrophic factors; and (2) the analysis of the regulation of neurotrophic factor and neuropeptide gene expression during development and in response to injury. Studies are underway to identify trophic factors produced in specific model systems, since recent evidence suggests that a family of nerve growth factors (NGF) exists, each specific for certain populations of neurons. An NGF-like factor increases in the cerebellum of the pcd mutant mouse as the Purkinje cells die out and astrocytes proliferate. MPTP-lesioned animals (both mice and monkeys) represent a Parkinsonian-like model in which changes in NGF and the related neurotrophic factors BDNF (brain-derived neurotrophic factor) and NT-3 (neurotropin-3) are being examined at the level of mRNA, protein and biologic activity. Since astrocytes can synthesize NGF, primary cultures of astrocytes are being used to determine factors which regulate NGF gene transcription as well as to assess production of these other potential trophic factors. Reactive astrocytes are prepared from regions affected by the various injuries and their production of trophic factors compared to that of control astrocytes. Potential neurotrophic functions for the neuropeptides, enkephalin and somatostatin, in early CNS development are being explored in several model culture systems.

At the same time, these injury models can be evaluated for changes in neuropeptide and/or neurotransmitter synthesis occurring in response to the lesions. One can derive an estimate of peptide turnover by combining measurements of the precursor mRNA, the precursor itself, and the peptide. Our studies have demonstrated that peptides are differentially regulated by such chronic drug treatments as reserpine, haloperidol, 6-hydroxydopamine or 5,7-dihydroxytryptamine. Work is in progress to determine the effects of CNS injury and recovery, including MPTP treatment, on various neuropeptides as well as such neurotransmitter synthetic enzymes as tyrosine hydroxylase and GAD, and the dopamine D_2 receptor in neurons, as well as on astrocyte and microglial gene expression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01NS00815-33DMN

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Metabolism of Complex Lipids of Nervous Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) Name, Title, Laboration, and Institute Affiliation

PI:	P G Pentchev, Ph.D.	Section Chief	DMN	NINDS
Others	R.O. Brady, M.D.	Chief	DMN	NINDS
	J.M. Quirk, M.S.	Biochemist	DMN	NINDS
	C. Roff, Ph.D.	Special Expert	DMN	NINDS
	E. Goldin, Ph.D.	Visiting Fellow	DMN	NINDS
	M. Comly, B.S.	Biologist	DMN	NINDS
	A. Cooney, B.S.	Biologist	DMN	NINDS

COOPERATING UNITS (if any)
Laboratory of Cellular and Developmental Biology, NIDDK; Laboratory of Biochemistry, Faculty of Medicine, Lyon-Sud, France

LAB/BRANCH
Developmental and Metabolic Neurology Branch

SECTION
Enzymology and Genetics; Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
8	6.0	2.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [X] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The metabolic defect in patients with Types C and D Niemann-Pick disease has been shown to be due to abnormal intracellular cholesterol homeostasis. The molecular lesion in these disorders results in: (1) failure to down-regulate LDL receptors on cell membranes; (2) lack of down regulation of HMGCoA reductase, a key enzyme in cholesterol biosynthesis; and (3) inability to up-regulate acyl cholesterol acyl CoA transferase, the enzyme that catalyzes the esterification of intracellular cholesterol. Tests have been developed and introduced into medical practice for the diagnosis of Types C and D Niemann-Pick disease and the identification of heterozygotes, and the prenatal diagnosis of these conditions.

We have linked the NP-C mutation to chromosome No. 18. Identification of the gene will enable us to assess direct DNA diagnosis and the final protein and gene localization studies. The Golgi apparatus has been shown to regulate lysosomal cholesterol transport. Characterization of the cholesterol transporter as identified by the NP-C mutation will provide the tools to begin to delineate the molecular mechanisms as well as cellular pathways of intracellular cholesterol transport. Armed with such information we will study cholesterol processing in normal cells and in pathogenic conditions represented not only by the NP-C cell but also by other cholesterol lipidotic states such as the atherogenic foam cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02162-19DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Compounds Analogous to Sphingolipids

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S.P. Miller, Ph.D.	Special Expert	DMN, NINDS
Others:	A. Boumendjel, Ph.D.	Visiting Fellow	DMN, NINDS

COOPERATING UNITS (if any)

Biochemistry and Molecular Biology Department, Georgetown University Medical Center

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Neurochemical Methology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project covers the synthesis of enzyme substrates and enzyme inhibitors which are used to study sphingolipid metabolism. The major ongoing project is the design and synthesis of inhibitors of sphingosine-1-phosphate lyase. This enzyme catalyzes the last step in the degradation of sphingosine: the cleavage of sphingosine phosphate to 2-hexadecenal and ethanolamine phosphate. The preparation of radiolabeled irreversible inhibitors would aid in the isolation and purification of the enzyme. This could lead to partial sequence determination, and ultimately to cloning the human or mouse gene. A second use for enzyme inhibitors would be to provide information on the biological effects of blocking sphingosine catabolism *in vivo*. Sphingosine has been reported to be an inhibitor of protein kinase C, and sphingosine-1-phosphate causes rapid translocation of calcium from intracellular stores. Blocking sphingosine-1-phosphate lyase would lead to accumulation of these two compounds, possibly causing profound changes in cellular regulation.

Our approach to the design of inhibitors is based upon the fact that sphingosine-1-phosphate lyase is a pyridoxal phosphate-dependent enzyme. We are synthesizing analogs of sphingosine that have these groups in the 2-position. 2-Vinyl diphydrazine and 2-hydrazino-dihydrosphingosine has already been prepared and characterized.

The 2-vinyl analog is efficiently phosphorylated by a rat liver cytosolic preparation that contains sphingosine kinase. Experiments are in progress to determine the extent of inhibition of sphingosine-1-phosphate lyase caused by treatment of cultured mammalian cells with 2-vinyl-dihydrosphingosine. Synthesis of 2-vinyl and 2-difluoromethyl analogs of the product ethanolamine phosphate are in progress. *In vitro* assays have been developed in our section for both the kinase and lyase reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02163-19DMN

PERIOD COVERED

October 1, 199 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Analytical Methods for Use in Research on Sphingolipidoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. P. Miller, Ph.D. Special Expert DMN NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Neurochemical Methodology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New analytical techniques were developed and used in enzymatic research and in clinical investigations of lipidoses.

The analysis of leukocytes and liver biopsy specimens from a patient with Gaucher's disease and a patient with Niemann Pick disease Type B continues. Both patients received orthotopic liver transplants for treatment of their sphingolipidoses. We are measuring the rate of reaccumulation of glucocerebroside and sphingomyelin, and are studying the post-transplantation changes in glucocerebrosidase and sphingomyelinase.

A patient with an unknown storage disorder was studied to determine the nature of the storage process. The patient exhibited multiple xanthomas of the aerodigestive track which were found to be composed primarily of triglyceride, cholesterol esters and cholesterol. Liver biopsy showed elevations of cholesterol, cholesterol ester and bis(monoacylglycerol)phosphate

Gaucher's disease is a lipidosis caused by a deficiency of the lysosomal enzyme, glucocerebrosidase. Significant changes occur in the bone marrow of patients with this disease. A study of the changes in the bone marrow lipids of Gaucher patients caused by disease progression or therapeutic intervention was concluded this year. The results of this study provide a biochemical basis for interpreting bone marrow changes seen by Quantitative Chemical Shift Imaging

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02453-13DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gaucher's Disease: Biochemical and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N Barton, M.D , Ph D	Chief, Clinical Care Unit	DMN	NINDS
Others:	R O. Brady, M.D.	Chief	DMN	NINDS
	G. Murray, Ph.D.	Special Volunteer	DMN	NINDS
	G Zirzow, B.S	Biologist	DMN	NINDS
	K. Oliver, M.S	Biologist	DMN	NINDS
	F. S Jin, M.D.	Special Volunteer	DMN	NINDS
	M. A McKee, M.D	Clinical Associate	DMN	NINDS
	T. Banerjee, M. D	Visiting Associate	DMN	NINDS

COOPERATING UNITS (if any)

Massachusetts Gen. Hospital, Dept of Orthopedic Surgery, Boston, MA: (H. Mankin, D. Rosenthal, S Doppelt); Children's Hospital, Washington, D C (P Guzetta)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Clinical Investigations & Therapeutics Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	4.5	2.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Extraordinarily gratifying success has been obtained with enzyme replacement therapy in patients with Gaucher's disease All patients who received macrophage-targeted human placental glucocerebrosidase had significant clinical benefit. The hemoglobin level rose in all patients, and within six months after initiation of therapy, the size of the spleen had decreased in all recipients The enzyme injections were well tolerated, and none of the patients became sensitized to the preparation. Patients who received the enzyme were able to resume activities such as work or school that they had been unable to carry out before enzyme replacement. The U S Food and Drug Administration has approved the use of macrophage-targeted glucocerebrosidase as specific therapy for patients with Type 1 Gaucher's disease The beneficial effect of enzyme replacement in patients with Gaucher's disease has been repeatedly confirmed by many independent investigators We have found that the quantity of enzyme that patients require to be maintained in good health is far less than that which is initially necessary to reverse the clinical and pathological manifestations of the disorder Patients with milder clinical signs of the disorder improve with smaller amounts of enzyme than that required by more severely affected individuals

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02664-09DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Neurogenetic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N Barton, M.D.,Ph D.	Section Chief	DMN	NINDS
Others:	R Brady, M.D	Chief	DMN	NINDS
	J. Higgins, M.D	Clinical Associate	DMN	NINDS
	C. Parker, M D	Clinical Associate	DMN	NINDS
	R. Schiffmann, M.D	Visiting Associate	DMN	NINDS
	M. A. McKee, M.D	Clinical Associate	DMN	NINDS
	T Bannerjee, M.D	Visiting Associate	DMN	NINDS
	P Pentchev, Ph D	Section Chief	DMN	NINDS

COOPERATING UNITS (if any)

Neuroimaging Branch, NINDS, and Laboratory of Molecular and Cellular Neurobiology, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Clinical Investigations and Therapeutics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	6.5	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[x] (a1) Minors
[ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have found that the use of cholesterol-lowering agents such as Lovastatin, cholestyramine and nicotinic acid reduces the quantity of cholesterol in the blood and in the liver of patients with Type C Niemann-Pick disease. We shall use this information to design a clinical efficacy trial to determine the effect of these agents on the rate of progression of neurologic signs in these patents. We have identified a new demyelinating disorder in young females and documented highly unusual magnetic resonance spectroscopic aberrations in these patients

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02731-07DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy of Inherited Enzyme Deficiencies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S Karlsson, M D , Ph D	Acting Chief, M&MG	DMN	NINDS
Others:	L Xu, M D ,Ph D	Visiting Associate	DMN	NINDS
	S Klupfel-Stahl	Special Volunteer	DMN	NINDS
	P Correll, Ph D	Special Volunteer	DMN	NINDS
	R Brady,M D	Chief	DMN	NINDS
	R Schiffmann, M D	Visiting Associate	DMN	NINDS
	D Freas, B S	Chemist	DMN	NINDS
	N Barton	Chief, CITS	DMN	NINDS

COOPERATING UNITS (if any)

Clinical Hematology Branch, NHLBI (Drs R Donahue and C Dunbar)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda Maryland 20892

TOTAL MAN-YEARS	PROFESSIONAL	OTHER
12.0	6.5	5.5

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gaucher's disease is an inherited disorder caused by a mutation of the gene for the enzyme glucocerebrosidase. The normal gene for this enzyme has been cloned by several laboratories. We have constructed high-titer, helper-free recombinant retroviruses containing this gene. We have shown that infection of cell lines from normal individuals and patients with Gaucher's disease with this retroviral vector results in increased glucocerebrosidase activity. The glucocerebrosidase gene has been transferred efficiently into progenitor cells and repopulating stem cells of mouse bone marrow, and is expressed at the RNA and protein level in the progeny of CFU-S multipotential progenitor cells following gene transfer. The gene has also been transferred efficiently into murine hematopoietic stem cells that can be used to repopulate secondary transplant recipients. The vector genome can be detected in all hematopoietic lineages and produces human glucocerebrosidase RNA in all hematopoietic tissues tested. High levels of human glucocerebrosidase are generated in hematopoietic tissues. The macrophages of these long-term reconstituted mice produce human glucocerebrosidase levels that are equivalent to the endogenous mouse enzyme levels. The human glucocerebrosidase gene has been introduced into human hematopoietic progenitor cells with a high degree of efficiency. Vector transduced hematopoietic progenitors from Gaucher's patients produce progeny cells with glucocerebrosidase enzyme values similar to those of normal individuals. A clinically acceptable supernatant infection protocol has recently been developed which can be used to correct the enzyme deficiency in hematopoietic cells from Gaucher patients following gene transfer into primitive hematopoietic cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02771-05DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modification of Growth Factor Genes by Gene Targeting

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	A Kulkarni, Ph.D.	Senior Staff Fellow	DMN	NINDS
Others:	S Karlsson, M.D., Ph D	Acting Section Chief	DMN	NINDS
	D Becker, B. S	Biologist	DMN	NINDS
	J. Higgins, M D.	Clinical Associate	DMN	NINDS
	C.-G Huh, Ph.D.	IRTA Fellow	DMN	NINDS
	M Sporn, M.D.	Chief	LC	NCI
	A Roberts, Ph D	Deputy Chief	LC	NCI
	A Geiser, Ph D.	Senior Staff Fellow	LC	NCI

COOPERATING UNITS (if any)

Laboratory of Chemoprevention, NCI

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
8.5	5.5	3.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene targeting by homologous recombination can be used to activate or inactivate cellular genes in eukaryotic cells. The objective of this project is to alter the functional status of genes that control growth and maturation of specific tissues and study the biological consequences of these molecularly defined alterations. To delineate specific developmental roles of transforming growth factor beta$_1$ (TGF-beta 1), we have disrupted its cognate gene in mouse embryonic stem cells by homologous recombination to generate TGF-beta 1 null mice. These mice do not produce detectable amounts of either TGF-beta 1 RNA or protein. After normal growth for the first two weeks, they develop a rapid wasting syndrome and die by three weeks of age. Pathological examination revealed an excessive inflammatory response with massive infiltration of lymphocytes and macrophages in many organs, but primarily in heart and lungs. Many lesions resembled those found in autoimmune disorders, graft-vs.-host disease, or certain viral diseases. This phenotype suggests a prominent role for TGF-beta 1 in homeostatic regulation of immune cell proliferation and extravasation into tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS02735-15 DMN

PERIOD COVERED

October 1, 1990 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generation of Widespread Single Cell Chimeras

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Kleinsmith, M.D., Ph.D.	Acting Chief, W&MG	DMN	NINDS
OTHER:	D. Press, B.S.	Chairman	DMN	NINDS
	A. Schleicher, M.D.	Chief	LCB	NINDS
	C. Nagaoka, Ph.D.	Sen. Scientist	LCB	NIDDK
	S. Shenker, M.D.	Staff Fellow	LCB	NIDDK

COOPERATING UNITS (if any)

Dept. of Biology, U. of S. Carolina, Prof. W. Dewey

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-MAN-YEARS	PROFESSIONAL	OTHER
3.0	2.5	.5

CHECK APPROPRIATE BOX(ES)

___ (a) Human Subjects ___ (b) Human Tissues X (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02816-04DMN

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Inhibitors of N-Myristoyltransferase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S. P. Miller, Ph.D	Special Expert	DMN	NINDS
Others: K.M.Neder, Ph.D	IRTA Fellow	DMN	NINDS
S. A. French, B.S	Chemist	DMN	NINDS

COOPERATING UNITS (if any)

Laboratory of Molecular Biology, DTTD, FDA
Cell Signaling and Oncogenesis Group, Clinical Pharmacology Branch, NCI

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Neurochemical Methodology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is the synthesis of new inhibitors of the enzyme myristoyl-CoA:protein N-myristoyltransferase (NMT). This enzyme catalyzes the covalent modification of specific proteins by acylating the amino group of N-terminal glycines with myristoyl-CoA. Biomedically important proteins which are myristoylated include oncoproteins such as p60 *src* and the *gag* polypeptide of HIV and other retroviruses. The goal of this project is to design and synthesize inhibitors of protein myristoylation that are active *in vivo* for testing as anti-retroviral agents. More than 39 analogs of the substrate, myristoyl-CoA, or of the myristoylated peptide product have been synthesized to date. These include compounds designed to be either competitive or irreversible inhibitors of NMT. In addition, another 12 compounds have been obtained from commercial sources. All have been tested in an *in vitro* NMT assay developed within this project. Among the new compounds synthesized in our section, two have been determined to be highly potent inhibitors of protein myristoylation. Both have IC_{50} values below 0.1 uM. The regions of our substrate- and product-analogs that are necessary for high-affinity binding to NMT are being identified and incorporated into future syntheses. Two other synthetic compounds have shown activity in the NCI *In Vitro* Primary Antitumor Screen. One compound has specificity against the renal cancer panel, while the other primarily inhibits the growth of colon cancer lines. Both have been selected by the NCI Biological Evaluation Committee for further testing in rodents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02843-02DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of the Etiology of Mucolipidoses IV

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R O. Brady, M D.	Chief	DMN	NINDS
Others:	E.Goldin, Ph.D.	Visiting Fellow	DMN	NINDS
	P G. Pentchev, Ph.D	Section Chief	DMN	NINDS
	N W Barton, M D , Ph D	Section Chief	DMN	NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Cellular and Molecular Pathophysiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The etiology of Mucolipidosis IV is currently unknown. We shall explore rational biochemical and cell biology leads to obtain insight into the pathogenesis and molecular abnormality in this hereditary disorder. Our principal goals are to develop accurate diagnostic and carrier detection tests for genetic counseling and realistic approaches to the therapy of this hereditary disorder

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02844-02DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Investigation of the Etiology of Batten's disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Calvin F. Roff, Ph D	Special Expert	DMN	
Others:	Peter G. Pentchev, Ph D	Section Chief	DMN	NINDS
	Roscoe O. Brady, M D	Branch Chief	DMN	NINDS

COOPERATING UNITS (if any)

Section on Receptor Biochemistry and Molecular Biology, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Molecular and Cellular Pathophysiology

INSTITUTE AND LOCATION

NINDS,NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The etiology of Batten's disease is unknown at this time. We intend to explore biochemical cell biological and molecular biological leads to other information on the pathogenesis and molecular abnormalities in this and closely related conditions. Our goals are to develop precise diagnostic tests and effective therapies for patients with these disorders

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS02845-02DMN

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Investigation of Enzyme Replacement Therapy in an Analogue of Human GM1-Gangliosidosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. O. Brady, M.D.	Chief	DMN	NINDS
Others:	G. J. Murray, Ph.D.	Special Volunteer	DMN	NINDS
	J.M. Quirk, M.S.	Biochemist	DMN	NINDS

COOPERATING UNITS *(if any)*

Surgical Neurology Branch, NINDS

LAB/BRANCH

Developmental and Metabolic Neurology Branch

SECTION

Enzymology and Genetics

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enzyme replacement therapy has been shown to be extraordinarily effective for patients with Type 1 (non-neuronopathic) Gaucher's disease. We now need to develop procedures to deliver useful amounts of enzymes to the brain in patients with hereditary metabolic storage disorders. We shall examine the effect of human placental beta-galactosidase on the amount of ganglioside GM1 in animal analogues of human generalized (GM1) gangliosidosis using a new intracerebral protein delivery system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01NS-02878-01

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models for Genetic Defects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	S. Karlsson, M.D ,Ph.D	Acting Chief, M&MGS	DMN	NINDS
OTHERS:	C.-G. Huh, Ph.D	IRTA Fellow	DMN	NINDS
	J. Higgins, M.D.	Medical Staff Fellow	DMN	NINDS
	A. Kulkarni, Ph.D	Senior Staff Fellow	DMNB	NINDS
	D. Becker, B.S.	Biologist	DMN	NINDS

COOPERATING UNITS (if any)

P. Loh, Section Chief, NICHD

LAB/BRANCH

Developmental and Metabolic Neurology

SECTION

Molecular and Medical Genetics

INSTITUTE AND LOCATION

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	4.5	0.5

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Gene targeting in embryonic stem (ES) cells is being used to inactivate (knock-out) genes and use the mutated ES cells to generate mice with a mutation at the targeted locus. We have targeted the pro-opioelanocortin (POMC) gene in embryonic stem cells in order to determine the influence of POMC during development and post-natally. The POMC targeted ES cells are now being used to generate chimeric animals that may carry the gene defect in the germ-line. Similarly, the cystatin C gene has been cloned and gene targeting constructs have been made in order to inactivate the cystatin C gene in ES cells. Cystatin-C mice will be made and the muated human cystatin C gene from a patient with hereditary cerebral angiopathy will be introduced into one of the constructs in order to make a mouse model for hereditary stroke.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02236-18 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diagnostic and Therapeutic Reevaluation of Patients With Intractable Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Susumu Sato, M.D	Chief, EEG Lab	OCD	NINDS
	William D. Gaillard, M.D.	Clinical Associate (SF)	ERB	NINDS
	Susan Bookheimer, Ph.D	Staff Fellow	ERB	NINDS
	Teresa Blaxton, Ph.D.	Staff Fellow	ERB	NINDS
	Laroy Penix, M.D	Senior Staff Fellow	ERB	NINDS

COOPERATING UNITS (if any)

EEG Laboratory, Office of The Clinical Director, NINDS

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[x] (a1) Minors
[ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Clinical Epilepsy Section is using a multimodality approach to evaluate patients with severe epilepsy, including simultaneous video and telemetered electroencephalographic (EEG) recording of seizures, daily determinations of antiepileptic drug serum concentrations, positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetoencephalography (MEG). A specific seizure diagnosis is established allowing each patient to be assigned to an appropriate research protocol and therapy. PET uses radiolabelled tracers to measure cerebral glucose metabolism, blood flow, and neurotransmitter distribution. Focal hypometabolism may underlie epileptogenic zones. During seizures, increased glucose utilization and blood flow are found MRI may show small structural lesions underlying PET hypometabolism even when computed tomography (CT) is normal. Further studies will elucidate the relation of metabolic and pathologic changes MEG may have the potential to accurately localize the subsurface origin of spikes. EEG provides little information on the spatial distribution of epileptiform discharges in cortical depths; MEG may be superior. Digital signal processing is being applied to data from multiple closely spaced electrode arrays. Comparison of invasive localization of epileptic foci using subdural electrodes and noninvasive evaluation is being performed. After surgery, patients are followed with serial clinical, neuropsychological, and electroencephalographic evaluation. Children with partial seizures are followed with serial PET scans to assess the development of hypometabolism in the epileptic focus. The effect of the ketogenic diet is also being studied

Seizures in kindled and post cardiac arrest audiosensitive rats are used to study patterns of neuronal damage and their relation to altered electrophsyiology. Somatostatin (SS) neurons are selectively lost in the dentate hilus of patients with longstanding temporal lobe epilepsy. These neurons are vulnerable to non-NMDA but not NMDA-mediated neurotoxicity in cell culture. NBQX, a non-NMDA antagonist, protected against loss of SS as well as neuropeptide Y (NPY)-containing neurons, while MK-801 protected only against the former. Paired-pulse inhibition was lost in both experimental groups. SS and NPY immunoreactive neurons may not be responsible for this type of inhibition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02318-16 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antiepileptic Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William H. Theodore, M.D.	Chief, CES	ERB	NINDS
Others:	Susumu Sato, M.D.	Chief, EEG Laboratory	OCD	NINDS
	William D Gaillard, M.D.	Clinical Associate (SF)	ERB	NINDS
	J. Robert Flamini, M.D	Visiting Associate (CA)	ERB	NINDS

COOPERATING UNITS (if any)

Office of The Clinical Director, NINDS

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[X] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuroimaging techniques are used to study the effect of antiepileptic drugs on cerebral glucose metabolism. In our most recent investigations, we found that valproic acid reduced cerebral blood flow (CBF) and glucose metabolism to the same degree as phenytoin (PHT) or carbamazepine (CBZ) but less than phenobarbital. The effect was most prominent in the thalamus, which may be related to the efficacy of valproic acid against absence seizures. Endogenous opiates have been implicated in the pathophysiology of epilepsy ^{18}F cyclofoxy, a naltrexone analogue, was used to image mu and kappa opiate receptors in patients with complex partial seizures. We found, in a few of the patients, increased opiate ligand binding ipsilateral to the epileptic focus, but no difference in the group as a whole. In the rat kainic acid model of epilepsy, bilateral decreases in dentate gyrus dynorphin immunohistochemical staining occurred despite unilateral cell loss on Nissel staining. No changes in met-enkephalin were found, supporting the hypothesis that kappa but not delta receptors are down-regulated.

We have evaluated the effect of drug withdrawal on seizure frequency in order to assess the presence or absence of transient exacerbations which could be distingushed from a simple loss of drug effect. This was clearly present in the case of phenobarbital and CBZ, but absent for PHT. For CBZ, rate of discontinuation was significantly related to seizure frequency. Neuropsychiatric disorders such as panic were increased during drug withdrawal. These data are important for clinical practice. A physician wishing to withdraw a drug known to cause a transient exacerbation during taper may be more likely to perservere when seizures increase

We have conducted two double-blind placebo controlled trials of felbamate, an experimental anti-epileptic drug, in patients with complex partial seizures and the Lennox-Gastaut syndrome, a severe childhood epileptic encephalopathy. Patients are entered into the complex partial seizure trial after they have been tapered off their other drugs for surgical monitoring. This process simplifies data collection and clinical screening for several potential trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS-02858-02 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological and Cognitive Studies in Epilepsy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William Theodore, M.D	Chief, CES	ERB	NINDS
Others:	William D. Gaillard, M.D.	Clinical Associate (SF)	ERB	NINDS
	Susan Bookheimer, Ph.D.	Staff Fellow	ERB	NINDS
	Teresa Blaxton, Ph.D.	Staff Fellow	ERB	NINDS

COOPERATING UNITS (if any)

Medical Neurology Branch

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Clinical Epilepsy Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have been performing imaging studies of language organization in normal controls and patients with epilepsy. Using positron emission tomography (PET), activation of cerebral blood flow (CBF) associated with word and object recognition, auditory comprehension, and phoneme, word, and sentence production are localized in the brain. Data from subdural stimulation, PET, and magnetic resonance imaging (MRI) are integrated using digital image processing techniques. The combined stimulation and PET data allow us to study the relationship between activation and disruption of cognitive activity, and to form more accurate concepts of the organization of cerebral function. These studies will elucidate the function of regions such as the basal temporal language area, which are of clinical importance when surgery for uncontrolled seizures is planned. Digital signal processing techniques are used to confirm anatomic localization of functional mapping. Using surface fitting algorithms, PET, CT, MRI, and subdural electrode positons are aligned. In PET experiments, rest conditions are averaged and subtracted from activated conditions, in order to reveal reigons of inreased blood flow during task performance. We found a high concordance between PET-CBF and subdural stimulation mapping using a number of different functional tets. This result shows the practicality of noninvasive preoperative functional brain mapping, and also demonstrates the close correlation of disruption and activation studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02732-07 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological Studies of Ion Channels in Cultured Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. Michael A. Rogawski, M.D , Ph.D. NES, ERB, NINDS

Others: D. M. Politi, M.D.,Visiting Associate, ERB, NINDS;T. Kokate, Ph.D. Visiting Fellow, NES, ERB, NINDS; T. R. Werkman, Ph.D., Visiting Fellow, NES, ERB, NINDS; K. Wayns, B.S., Lab. Tech., NES, ERB, NINDS; S. Donevan, Ph.D., Visiting Fellow, NES, ERB, NINDS, R. P. Irwin, M.D., CNB, NIMH; S. Subramaniam, M.D., Ph.D., Visiting Associate, NES, ERB, NINDS; J. Rho, M.D. Medical Staff Fellow, NES, ERB, NINDS; C. Hough, Ph.D , Biological Psychiatry Branch, NIMH; D-M. Chuang, Ph.D., BPB, NIMH

COOPERATING UNITS (if any)

Kanazawa University, Japan; Department of Physiology, University of Maryland School of Medicine

LAB/BRANCH

Epilepsy Research Branch

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 4.5	PROFESSIONAL: 4.5	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Whole-cell voltage-clamp and single channel recording techniques were used to study drug interactions with N-methyl-D-aspartate (NMDA) and non-NMDA receptor coupled cation and $GABA_A$ receptor-coupled Cl^- channels in cultured hippocampal neurons and with voltage-dependent K^+ channels in fibroblasts transfected with K^+ channel genes. The aim of this work was to explore new strategies for the rational development of antiepileptic drugs based upon their interaction with neuronal ion channel systems. Work focused in the following areas: (i) characterization of the actions of felbamate on NMDA and $GABA_A$ receptors; (ii) interaction of remacemide and its des-glycinated metabolite (FPL 12495) with NMDA receptors; (iii) studies on 2,3-benzodiazepine non-NMDA antagonists (GYKI 52466 analogs); (iv) interaction of a novel scorpion toxin (Tityustoxin-Kα) with the cloned Kv1.2 K^+ channel; (v) interaction of benzopyran K^+ channel openers with voltage-dependent K^+ channels in cultured hippocampal neurons; (vi) block of NMDA receptors by polyamines; and (vii) neurosteroid modulation of $GABA_A$ receptors. In addition, studies were carried out on the interaction of the anticonvulsant carbamazepine with NMDA receptor responses in cultured cerebellar granule cells using the Ca^{2+}-sensitive indicator Fura-2. Felbamate, a promising new antiepileptic agent, was found to inhibit NMDA responses and potentiate GABA responses (via a barbiturate-like effect) at clinically relevant concentrations. This novel combination of actions may account for felbamate's unique clinical profile. Remacemide, an antiepileptic undergoing clinical investigation, is des-glycinated *in vivo* to form 1,2- diphenyl-2-propylamine (FPL 12495). We observed that this metabolite produces a stereoselective open channel block of NMDA receptors, supporting the view that remacemide may serve as a prodrug for an NMDA antagonist. We have previously demonstrated that the 2,3-benzodiazepine GYKI 52466 is a potent antagonist of non-NMDA (AMPA/kainate)-type glutamate receptor responses in cultured hippocampal neurons. We now show that certain structural modifications of GYKI 52466 at position 3 can enhance blocking potency. The parallel increase in potency of GYKI 53655 in blocking AMPA/kainate receptor currents and in seizure protection provides further evidence that the anticonvulsant activity of GYKI 52466 and its analogs is due to antagonism of AMPA/kainate receptors. Noncompetitive AMPA/kainate antagonists (i.e., GYKI 52466) could offer advantages over competitive antagonists in treating seizures, particularly under conditions where high glutamate levels would render the competitive antagonists relatively ineffective.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02733-07 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Excitability Properties of Enzymatically Dissociated CNS Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Michael A. Rogawski, M.D., Ph.D. Chief, NES, ERB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been subsumed under preexisting project Z01 NS 02732-07 ERB.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02772-06 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Uncompetitive NMDA Antagonists as Anticonvulsants

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Rogawski, M.D., Ph.D.	Chief, NES	ERB	NINDS
Others:	Shun-ichi Yamaguchi, Ph.D	Psychologist, NES	ERB	NINDS
	Izyaslav Lapin M.D.	Visiting Scientist, NES	ERB	NINDS
	Tushar Kokate, Ph.D.	Visiting Fellow, NES	ERB	NINDS
	Kenner C. Rice, Ph.D.	Chief, Lab. Medicinal Chemistry		NIDDK
	Duangchan Uyakul, Ph.D.	Lab. Analytical Chemistry		NIDDK
	Lewis K. Pannell, Ph.D	Lab. Analytical Chemistry		NIDDK

COOPERATING UNITS *(if any)*

Neurogen Corporation, Branford, CT; Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	2	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Plasma levels of ADCI (5-aminocarbonyl-5H-dibenzo[a,d]cyclohepten-5,10-imine), a low-affinity uncompetitive NMDA antagonist currently under development for use in epilepsy therapy, were determined using gas chromatography-mass spectroscopy following acute and chronic dosing in mice. At anticonvulsant doses, plasma levels were achieved that were within the range that produce substantial blockade of NDMA receptors (as determined in *in vitro* experiments). Following chronic (2 wk) administration of ADCI, mice exhibited tolerance to the anticonvulsant effects of the drug that could be overcome by raising the dose. This tolerance appeared to be due to induction of metabolism and not to pharmacodynamic factors since the blood levels achieved with the higher anticonvulsant doses in tolerant animals corresponded closely to the levels achieved in naive animals receiving an anticonvulsant dose. ADCI was resolved into its optical enantiomers. (+)-ADCI was approximately twice as potent an anticonvulsant in the maximal electroshock test as (-)-ADCI and had a somewhat higher therapeutic index, suggesting that the (+)-enantiomer may be more appropriate for further clinical development than the racemate. Drug discrimination studies in rats trained to discriminate dizocilpine from saline indicated that ADCI does not substitute for dizocilpine and that other low-affinity uncompetitive NMDA antagonists only weakly substitute for the drug. These results suggest that low-affinity uncompetitive NMDA antagonists may have a superior side effect profile than conventional NMDA antagonists, and support the potential utility of this class of compounds in epilepsy therapy. Dopamine receptor blockade with haloperidol, cis-flupenthixol (a combined D1 and D2 antagonist) or a combination of raclopride (a selective D1 antagonist) and SCH 23390 (a selective D2 antagonist) was found to attenuate the stimulation of locomotion induced by dizocilpine, indicating that dopamine receptor antagonists might be useful in preventing the adverse behavioral effects of uncompetitive NDMA antagonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02877-01 ERB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preclinical Evaluation of Novel Anticonvulsant Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Michael A. Rogawski, M.D., Ph.D.	Chief, NES,ERB, NINDS
Others:	Shun-ichi Yamaguchi, Ph.D.	Psychologist, NES, ERB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Epilepsy Research Branch, CNP, DIR

SECTION

Neuronal Excitability Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 1	PROFESSIONAL: 1	OTHER: 0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation of novel antiepileptic drugs in animal seizure models is being carried out as a complement to studies on the interaction of these drugs with ion channels in in vitro systems. The anticonvulsant activities of a noncompetitive (GYKI 52466: 1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H- 2,3-benzodiazepine) and a competitive (NBQX: 2,3-dihydroxy-6-nitro-7- sulfamoyl-benzo[f]quinoxaline) non-NMDA (AMPA/kainate) excitatory amino acid antagonist were compared in the maximal electroshock (MES) seizure test and various chemoconvulsant models. Both antagonists were protective in the MES and pentylenetetrazol tests. GYKI 52466 was also protective against seizures and lethality induced by 4-aminopyridine, kainate and AMPA, but not by NMDA, whereas NBQX was ineffective in these chemoconvulsant tests. Both GYKI 52466 and NBQX produced motor impairment at doses similar to those that were protective in the MES test. We conclude that under some circumstances, noncompetitive AMPA/kainate antagonists could offer advantages over competitive antagonists in the treatment of seizures. However, neurological toxicity is an obstacle to the potential clinical use of both classes of agents. The effectiveness of AMPA/kainate antagonists in standard anticonvulsant screening models suggests that such compounds could have utility in epilepsy therapy. Noncompetitive AMPA/kainate antagonists, like GYKI 52466, may offer advantages over competitive antagonists in certain seizure types, especially those associated with high synaptic levels of glutamate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02263-17 ETB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Pharmacological Studies of Dopamine Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: David R. Sibley, Ph.D Chief, Molecular Neuropharmacology Section ETB/NINDS

Others: Frederick J. Monsma, Jr., Ph.D., Senior Staff Fellow; Jean E. Lachowicz, Ph.D., PRAT Fellow; Tom R. Hollon, Ph.D., IRTA Fellow; Brian N. Atkinson, Ph.D., IRTA Fellow; Steven I. Max, Ph.D., IRTA Fellow; Loyd H. Burgess, Ph.D, IRTA Fellow; Yong Shen, Ph.D., Visiting Fellow; Li-Juan Zhang, Ph.D., Visiting Fellow; Antonio M. Gonzalez, Ph.D., Fogarty Fellow; Sara Fuchs, Ph.D., Guest Researcher, ETB/NINDS

COOPERATING UNITS (if any)

Lab Cell Biol., NIMH; Lab of Mamm. Genes & Devel., NICHD; Neurosci Inst, Chicago Med Scl; Psych. Dept, Wayne St Univ; Psych Dept, Seattle VAMC, Psych. Dept, Case West Res Univ; UCLA Med Ctr, CA

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Molecular Neuropharmacology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 8.35	PROFESSIONAL: 8.35	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
  [ ] (a1) Minors
  [ ] (a2) Interviews

[ ] (b) Human tissues

[x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long-term goal of this project is the characterization of neurotransmitter receptor-mediated information transduction and its regulation, across neuronal membranes. The primary, but not exclusive, model systems under investigation are those for dopamine (DA) receptors. In order to characterize DA and related receptors at the biochemical and molecular levels and study their regulation, there are two major interrelated lines of research which are ongoing: 1) investigation of the cell biology, function and regulation of the receptors at the protein level; and 2) the molecular cloning of the receptor genes and investigation of gene structure and regulation in normal and pathophysiological states.

1. Cell Biology and Regulation of DA Receptors: Characterization of the functional and regulatory properties of D_1 and D_2 DA receptors on various neuroblastoma and cDNA-transfected cell lines was continued. The D_{1A} receptor was shown to undergo agonist-induced desensitization in CHO cells that is partially cAMP-mediated and involves both functional uncoupling and down-regulation of the receptors. Both short and long isoforms of the D_2 receptor (D_{2S} and D_{2L}) were also shown to undergo desensitization in CHO cells in response to agonist treatment. The D_{2S} receptor was also down-regulated by agonist treatment whereas the D_{2L} receptor was paradoxically up-regulated. Both D_2 receptor isoforms were expressed in NG108-15 neuroblastoma cells and shown to couple to K^+ channels, albeit through different G proteins. The D_3 receptor was also demonstrated to couple to K^+ channels in the NG108-15 cells.

2. Molecular Cloning of DA and Other Receptors: The distribution of the D_{1A} and D_{1B} receptors were mapped in the kidney. Both D_1 receptor subtypes were sequenced in the spontaneous hypertensive rat (SHR) which exhibits defective kidney D_1 receptors. No differences in sequence were found in comparison to control rats. Work continued on the cloning of a third "D_1-like" receptor which apparently is linked to the stimulation of phosphatidylinositol turnover and mobilization of calcium. Transgenic "knock-out" experiments for several of the DA receptor subtypes were initiated. Two completely novel serotonin receptors were cloned and expressed. These were designated the 5-HT6 and 5-HT7 serotonin receptor subtypes. Several other cDNA clones encoding putative "orphan" G protein-linked neurotransmitter receptors were identified.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02826-03 ETB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Regulation of Transmitter Receptor Genes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, Title, Laboratory, and Institute Affiliation)

PI:	M. Maral Mouradian, M.D.	Head, Genetic Pharmacology	ETB, NINDS
Others:	Takashi Minowa, Ph.D.	Visiting Fellow	ETB/NINDS

COOPERATING UNITS (if any)

Dept. Physiology, Uniformed Services Univ. of the Health Sciences; Sect. Pediatric Nephrology, Georgetown Univ. Med. Ctr.; Dept. Pharmacology, Univ. Nebraska, Omaha, NE.

LAB BRANCH

Experimental Therapeutics Branch

SECTION

Genetic Pharmacology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

The main discoveries of the Genetic Pharmacology Unit during FY 93 were the following: (1) Transcriptional regulation of the D_{1A} dopamine (DA) receptor gene: We had previously localized the main enhancer of the human D_{1A} gene between nucleotides -1342 and -1102 relative to the first ATG codon. In FY 93, we found that most of this enhancer activity is between -1154 and -1137 and some also between -1197 and -1155. We determined that this enhancer interacts with multiple nuclear proteins as complexes to activate the promoter. Although the sequences protected by these complexes include AP2 binding sites, we discovered that AP2 is not involved in positively modulating the basal expression of the D_{1A} gene. The complex at the main enhancer includes at least two proteins one of which is antigenically related to Sp1 or is Sp1 itself. (2) Promoter analysis of the D_2 DA receptor gene: During FY 93, we focused on the silencer of the rat D_2 gene which we had previously localized between nucleotides -217 and -75. An Sp1 consensus sequence and a TGGG repeat in this silencer interact with a complex of two proteins one of which is Sp1. We also discovered that most of the silencing activity is actually localized between -160 and -135 although thus far no *in vitro* DNA/protein interaction has been detected in this region. (3) Analysis of the 5' flanking region of the D_3 DA gene: Toward our efforts to characterize the 5' flanking region of the rat D_3 gene, we cloned and sequenced its true exon I. We found that unlike the D_2 gene, the D_3 exon I is only 50% G + C rich, although the two genes are highly homologous in their coding region. Using the new sequence information, we cloned a genomic fragment including the upstream region of the D_3 gene. (4) Genetic regulation of the rat BDNF gene: During our efforts to clone the most upstream exon in the rat BDNF gene, we discovered that there are at least five different first exons alternatively spliced with a common coding exon II. Each of these first exons appear to be transcribed from an alternate promoter that may be active in a brain region-specific manner. (5) Regulation of POMC gene transcription: We localized a CRH responsive region between -141 and -106 relative to transcription start site in the mouse POMC gene. The second messenger systems transducing this signal transcription coupling appeared not to exert their actions solely through PKA or PKC. (6) Protection against neuronal degeneration. We discovered that an adenosine A_1 agonist protects against and restores MPTP-induced striatal dopamine depletion in rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02139-19 ETB

PERIOD COVERED
October 1, 1992 through September 31, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pharmacology and Physiology of the Substantia Nigra and Basal Ganglia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Judith R. Walters, Ph.D.	Section Chief	ETB/ NINDS
Others:	Debra Bergstrom, Ph.D.	Pharmacologist	ETB/NINDS
	Michael Twery, Ph.D.	Senior Staff Fellow	ETB/NINDS
	Kai-Xing Huang, Ph.D.	Special Volunteer	ETB/NINDS
	Lisa Thompson, Ph.D.	Staff Fellow	ETB/NINDS

COOPERATING UNITS (if any)
Clinical Pharmacology Section, Experimental Therapeutics Branch

LAB/BRANCH
Experimental Therapeutics Branch, CNP

SECTION
Neurophysiological Pharmacology Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, MD 20892

TOTAL STAFFYEARS: 6.0	PROFESSIONAL: 5.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) D_1 and D_2 Dopamine (DA) Receptors in Basal Ganglia - *in vitro* Studies: Studies in striatal slices were undertaken to investigate mechanisms which might account for different effects of D_1 agonists on striatonigral activity in normal, 6-OHDA-lesioned, and reserpine treated rats. Experiments utilizing intracellular recording techniques find, following DA cell lesion, that fenoldopam's effects on striatal neuronal excitability are not altered, but SKF 38393 exhibited both excitatory and inhibitory effects. The D_1 antagonist SCH 23390 failed to reverse SKF 38393-induced changes in excitability indicating they are not mediated through D_1 receptors; results raise concern about use *in vitro* neurophysiological studies of SKF 38393 as a prototypic D_1 agonist. In contrast, SKF 38393 and fenoldopam induced comparable changes in phosphoinositide accumulation in striatal slices from control and 6-OHDA-lesioned rats; this accumulation is greater after DA cell lesion.

(2) D_1 and D_2 Receptors in Basal Ganglia - *in vivo* Studies: Whether the lesion alters the steady-state levels of striatal D_2 mRNA remains controversial. Solution hybridization/ribonuclease protection assays found no significant differences in absolute counts for D_2 long or D_2 short mRNAs or in ratios of D_2/β-actin mRNAs between lesioned and unlesioned striata in rats studied 2, 4, 8 or 19 weeks after lesion, indicating that postsynaptic changes induced by DA denervation are not associated with alterations in steady-state levels of D_2 mRNA.

(3) Effects of DA Agonists - Subthalamic Nucleus: Examination of effects of excitatory amino acids and DA on the activity of subthalamic neurons using extracellular single unit recording techniques showed that blockade of NMDA or AMPA receptors had no significant effect on average on the firing of subthalamic neurons. The DA agonist, apomorphine, significantly increased the firing of subthalamic neurons, effects unexpected in light of current models of basal ganglia organization that predict increased inhibitory pallidosubthalamic activity following DA agonist administration.

(4) Effects of DA Agonists - DA Cells: Agonists were ranked for relative potency at D_2 and D_3 receptors and examined for ability to inhibit DA cell firing. Although D_2 receptors are expressed in greater numbers by DA cells, to date potency at D_3 receptors correlates better with the ED_{50} for DA cell inhibition of these agonists.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02265-17 ETB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology, Biochemistry and Physiology of Central Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Thomas N Chase, M D Chief ETB/NINDS

Others: Jeff Anderson, PhD, IRTA Fellow; Robert Boldry, PhD, IRTA Fellow; Daniele Bravi, M.D., Special Volunteer; Thomas M. Engber, PhD, Senior Staff Fellow; Stella Papa, M.D., Visiting Fellow; Christopher Randolph, PhD, Senior Staff Fellow; John Roberts, M.D., Clinical Associate; Young Sohn, M D , Special Volunteer

COOPERATING UNITS (if any)

Georgetown Univ ; Hosp De La Salpetriere, Paris; NIMH; NIDCD; NIA; NIDR; Univ. Pavia, Italy; Royal Ottawa Hosp , Canada

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Clinical Pharmacology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 10.0	PROFESSIONAL: 8.0	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [X] (b) Human tissues [ ] (c) Neither

[X] (a1) Minors

[X] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) Wearing-off phenomena, generally the initial motor complication in levodopa-treated Parkinsonian patients, have been attributed solely to the loss of dopamine (DA) storage capacity as a consequence of dopaminergic neuron degeneration. However, recent preclinical studies cast doubt on this view, since the duration of the motor effects of a single levodopa dose in rats rendered Parkinsonian by a fixed DA system lesion decrease during the course of daily levodopa therapy. Preliminary observations in Parkinsonian patients treated with the direct DA agonist, apomorphine, further support the view that postjunctional alterations resulting from intermittent levodopa treatment contribute substantially to the pathogenesis of wearing-off fluctuations. These changes appear relatively plastic, since a continuous infusion of levodopa for 10 days prolongs levodopa's duration of action by 30% and a 3-month infusion of lisuride, a DA agonist, does so by over 90%.

(2) Evidence in support of presynaptic mechanisms operating to compensate for the loss of DA neurons in Parkinsonian patients derives from clinical studies showing the interval between the injection of levodopa and its peak antiparkinsonian response declines as disease severity advances. No change in this interval occurs with apomorphine which acts by directly stimulating postsynaptic receptors. Preliminary observations now suggest that a COMT inhibitor may substantially prolong the antiparkinsonian action of levodopa without increasing adverse effects associated with hyperdopaminergic stimulation.

(3) Extrapyramidal motor function appears potently regulated by glutamatergic mechanisms. Intermittent levodopa administration to Parkinsonian rats, upregulates D_2 DA receptor-mediated functions and down-regulates those mediated by D_1 receptors. Resultant funneling of striatal output through the D_2 pathway could contribute to the pathogenesis of motor complications. Acutely administered MK-801, a selective NMDA antagonist, normalizes the functional changes in both the D_1 and D_2 systems; drugs that block AMPA glutamate receptors do not have this effect. Indeed, AMPA and NMDA antagonists exert opposite effects on catalepsy induced by dopaminergic antagonists.

(4) The hypothesis that glutamate replacement might confer symptomatic benefit to Alzheimer's disease patients whose cortical glutamatergic projections have degenerated, was evaluated by administration of cycloserine. This partial indirect NMDA agonist had no consistent effect on cognitive function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02667-09 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Analysis of Involuntary Movements

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director		OCD	DIR	NINDS
		Chief	HMCS	MNB	DIR	NINDS
Others:	Camilo Toro, M.D.	Visiting Associate	HMCS	MNB	DIR	NINDS
	Jau-Shin Lou, M.D., Ph.D.	Visiting Associate	HMCS	MNB	DIR	NINDS
	A. Pascual-Leone, M.D., Ph.D.	Visiting Associate	HMCS	MNB	DIR	NINDS
	Barbara Karp, M.D.	Chief, Consultation Service		OCD	DIR	NINDS
	Josep Valls-Sole, M.D., Ph.D.	Visiting Associate	HMCS	MNB	DIR	NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	0.8	0.7

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Involuntary movements have often been difficult to classify clinically. Clinical and physiologic analysis of a continuing series of patients has led to new classifications and pathophysiologic insights.

Patients with myoclonus have been studied to seek further understanding of this confusing involuntary movement. Detailed studies are in progress on the opsoclonus-myoclonus syndrome and on the phenomenon of negative myoclonus. Analysis is ongoing of the physiology of periodic movement in sleep.

Extensive clinical and physiologic studies have continued in patients with palatal tremor (myoclonus). We have further data confirming the division of these patients into two groups, essential and symptomatic.

A study of movement-related cortical potentials in patients with dystonia (hand cramps) has revealed an abnormality of cortical activation. This has been confirmed in additional studies with event-related desynchronization of the EEG.

Motor performance in patients with dystonia (hand cramps) has shown a deficit in a task with rhythmic sequential movements. Patients tend to press the keys longer and their performance deteriorates with time.

We have studied five patients with stiff-man syndrome in attempts to characterize the spinal and supraspinal mechanisms responsible for the generation of symptoms. Abnormalities of reflex mechanisms including lack of vibratory inhibition of H-reflex and abnormalities of reciprocal inhibition of the H-reflex were found in all patients indicating a dysfunction of normal inhibitory mechanisms involved in muscle relaxation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02669-09 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Analysis of Voluntary Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Mark Hallett, M.D.	Clinical Director		OCD	DIR	NINDS
		Chief	HMCS	MNB	DIR	NINDS
Others:	Camilo Toro, M.D.	Visiting Associate	HMCS	MNB	DIR	NINDS
	Thomas Zeffiro, M.D.,Ph.D.	Sr. Staff Fellow	HMCS	MNB	DIR	NINDS
	Steve Grill, M.D.	Clinical Associate	HMCS	MNB	DIR	NINDS
	Jau-Shin Lou, M.D.	Clinical Associate	HMCS	MNB	DIR	NINDS
	Plamen Gatev, M.D.	Special Volunteer	HMCS	MNB	DIR	NINDS

COOPERATING UNITS (if any)

Department of Rehabilitation Medicine, Clinical Center
Department of Nuclear Medicine, Clinical Center

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.6	6.3	2.3

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of voluntary movement focused on the role of the cerebellum. One issue was the contribution of the cerebellum to coordination. The results seem to indicate that the cerebellum is critical for the coordination of multijoint movement. One role of the cerebellum appears to be in the control of force. A second issue is the role of the cerebellum in motor learning. In tasks of motor learning, it has been demonstrated with additional studies that patients with cerebellar disturbances have difficulty with adaptation learning. A third issue is the role of the cerebellum in kinesthesia, the sense of movement. Results show a deficit in appreciation of velocity and duration in patients with cerebellar deficits.

Using O-15 labelled water as a marker for cerebral blood flow in positron emission tomography (PET) studies , we have been working on methods for improved anatomic correlation of regions of metabolic change by superimposing the PET image onto an MRI image. In studies of PET and functional MRI, we have shown plasticity of the motor cortex with transient deafferentation of a limb with an ischemic block.

Studies of movement-related cortical potentials have focused on identifying dipoles for the generation of the different components and the development of techniques for measuring event-related desynchronization and coherence analysis of the EEG. The dipoles have been compared with areas of activation with PET and an excellent correlation has been found. Studies have been completed in patients with dystonia that show reduction in amplitude of some EEG components. Studies in the Biomechanics Laboratory of the Department of Rehabilitation Medicine have focused on the control of balance and gait. A study is in progress of gait in patients with cerebellar disorders . Other studies are being done of balance in patients with cerebellar deficits. Studies are ongoing recording muscle spindle activity during voluntary movement and passive stretch. A therapeutic trial of buspirone in cerebellar patients showed some improvement in those mildly affected

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02711-08 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Utility and Physiology of Botulinum Toxin for Involuntary Movement Disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Mark Hallett, M.D.	Clinical Director		OCD	DIR	NINDS
		Chief	HMCS	MNB	DIR	NINDS
Others:	Barbara I. Karp, M.D	Chief, Consultation Service		OCD	DIR	NINDS
	Stephen Grill, M.D., Ph.D.	Clinical Associate	HMCS	MNB	DIR	NINDS
	Jau-Shin Lou, M.D., Ph.D.	Clinical Associate	HMCS	MNB	DIR	NINDS

COOPERATING UNITS *(if any)*

Speech Pathology Unit, NIDCD

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have been studying the efficacy of local injections of botulinum toxin for the treatment of different types of focal dystonias. Botulinum toxin injected in small doses directly into muscle binds to the neuromuscular junction, and inactivates it for approximately three months. We have also used botulinum toxin to study the physiology of focal dystonias.

Studies of the utility of botulinum toxin are being carried out in writer's cramp (and its varients such as pianist's cramp) in open-label and double-blind trials. Treatment appears effective at least in the short-term. Longer follow-up on our patients showed that 49% of patients find botulinum toxin injections of persistent benefit. Patients who continued treatment were frequently women with nonlocalized symptoms or dystonic cramp, and a long duration of benefit.

Five patients with arm tremor have been treated to date. One patient, with dystonia and essential tremor, has had an excellent response. Three patients have had partial benefit, and one patient has had no improvement.

We are conducting a phase I trial of botulinum toxin type F to see if this will benefit patients who have developed antibodies to type A. It appears to have similar efficacy and side effects to type A, although the duration of action is slightly less

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 NS [illegible]

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Noninvasive Stimulation of Human Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Mark Hallett, M.D.	Clinical Director		OCD	NINDS
		Chief	HMCS	MNB	NINDS
Others:	Leo Cohen, M.D.	Visiting Scientist	HMCS	MNB	NINDS
	A. Pascual-Leone, M.D., Ph.D.	Clinical Associate	HMCS	MNB	NINDS
	Joachim Brasil-Neto, M.D.	Visiting Fellow	HMCS	MNB	NINDS
	Josep Valls-Solé, M.D., Ph.D.	Visiting Associate	HMCS	MNB	NINDS
	Eric Wassermann, M.D.	Clinical Associate		OCD	NINDS

COOPERATING UNITS (if any)

Speech and Voice Pathology Unit, NIDCD

LAB/BRANCH

Medical Neurology Branch, DIR, DIR

SECTION

Human Motor Control Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: [illegible] PROFESSIONAL: [illegible] OTHER: [illegible]

CHECK APPROPRIATE BOX(ES)

- [X] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are using new methods for the noninvasive stimulation of the human cortex. Stimulation can be with a high-voltage electrical pulse or with magnetic stimulation. One purpose is to use these methods for noninvasive localization of different parts of the human cortex, including motor cortex, sensory cortex and language cortex. Another purpose is to study cortical physiology in different disease states.

We have continued to make advances in understanding the technical aspects of magnetic stimulation, defining the optimal methods to map different body part representations in the motor cortex. We have compared the site of stimulation with the area of the brain activated in positron emission tomography (PET) scans by mapping the results of both studies onto the same MRI; such correlation is excellent. Extensive effort has been devoted to the study of the inhibitory effects of brain stimulation. Silent periods are much longer with magnetic stimulation than with electrical stimulation, suggesting that most of the effect comes from within the motor cortex. Topographic maps of the [illegible] of a muscle are generally [illegible] to those of the motor evoked potential of the same muscle. [illegible] ipsilateral muscles [illegible] the same muscle [illegible] ipsilateral muscle [illegible] contralateral muscle. [illegible] reorganization of the motor cortex [illegible] we have shown that this effect is [illegible] at the cortical level.

Magnetic stimulation is able to detect and define [illegible] motor representations [illegible] with the acquisition of [illegible] Braille reading [illegible]. These studies suggest that there is [illegible] which is determined [illegible].

We have found that following exercise to the point of fatigue the compound muscle action potential of a muscle to peripheral nerve stimulation remains unchanged; however, the [illegible] of the same muscle to magnetic stimulation decreases in amplitude. This shows that postexercise fatigue arises in the central nervous system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02792-05 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Investigations of Human Cognition and Mood State

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J. Grafman, Ph.D., Chief, CNS, MNB, NINDS

Others:

R. Johnson, Jr., Ph.D., Psychol., CNS, MNB, NINDS
P. Nichelli, M.D., Vis. Scientist, CNS, MNB, NINDS
I. Appollonio, M.D., Spec. Vol., CNS, MNB, NINDS
M. Hallett, M.D., Chief, MNB, NINDS
V Goel, Ph.D., Vis. Fellow, CNS, MNB, NINDS
L. Rueckert, Ph.D., IRTA Fellow, CNS, MNB, NINDS
A. Partiot, M.D., Spec. Vol., CNS, MNB, NINDS
A. Lee, M.D., Spec. Vol., CNS,MNB

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current studies in the Cognitive Neuroscience Section focus on amnesia, thinking, neurolinguistics, social cognition, and visual processing. Both single-case and group design studies are used. Normal controls, inpatients and outpatients are evaluated. Memory is studied in experiments focusing on implicit and explicit retrieval, priming, autobiographic recall, discourse processing, naming and word retrieval, and categorization tasks. Reasoning and problem-solving are studied in experiments focusing on planning, syllogisms, analogical thinking, and schema organization. Dyslexia, dysgraphia, and dysnomia, are studied in experiments focusing on single word reading and writing, lexical decision, associative and semantic priming, and similar tasks. Emotions, impression and preference formation, and social judgment are studied in experiments focusing on judgment of interpersonal behavior, word association, and mood state. Finally, visual information processing is studied, beginning with experiments examining spatial frequency contrast-sensitivity, object recognition, and visual categorization. Although developing theoretically valid and testable models of cognitive processing is the primary aim of the Section, there is also a strong effort to relate the profile of cognitive deficits in patients to lesion location in order to topographically map the components of cognitive processing to brain regions and systems. Pharmacologic challenge and infusion studies are planned to evaluate the dissociability of hypothesized components of memory processing. MRI functional stimulation and PET scan studies are employed to examine whether plans are processed in a unique brain location.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02793-05 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cognitive Neuroscience

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Jordan Grafman, Ph.D	Chief, CNS, MNB, NINDS
Others:	A. Salazar, M.D.	Department of Neurology, Walter Reed Army Med. Ctr.
	S. Rao, Ph.D	Dept. of Neurology, Medical College of Wisconsin
	F. Boller, Ph.D.	INSERM U. 324 Centre Paul Broca, Paris, France
	Y. Agid, M.D.	INSERM U. 289 Hopital Salpetriere, Paris, France
	A. Sirigu, Ph. D.	INSERM U. 289 Hopital Salpetriere, Paris, France
	B. Dubois, M.D.	INSERM U. 289 Hopital Salpetriere, Paris, France
	*	

COOPERATING UNITS *(if any)*

Walter Reed Army Medical Center, Wash, DC; National Naval Medical Center, Bethesda, MD; Centre Paul Broca, Paris, France; Hopital Salpetriere, Paris, France; Hospital Clinicas, Montevideo, Uruguay; **

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Memory and cognition are studied in experiments focusing on representational knowledge, working memory, priming, procedural learning, number processing and calculation, autobiographical memory, visual attention, naming, and categorization. Normal subjects and patients with progressive dementia, focal lesions, and psychiatric disorders are studied. New studies focusing on the composition of mental structures in the frontal lobes have just begun.

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*Continued:

J. Hallenbeck, M.D.	Chief, Stroke Branch, NINDS
E. Zaidel, Ph.D	Dept. of Psychology, UCLA, Los Angeles, CA
C. Junque, Ph.D	Dept. of Neurology, Hosp. St. Pau, Barcelona, Spain
J. Hendler, Ph.D.	Dept. of Computer Science, University of Maryland
K. Holyoak, Ph.D.	Dept. of Psychology, UCLA, Los Angeles, CA

** Medical College of Wisconsin, Milwaukee, Wisconsin; National Institute of Mental Health, NIH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02794-05 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Event-Related Potential Studies of Normal and Abnormal Cognitive Processing

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Ray Johnson, Jr., Ph.D.	Psychologist	CNS	MNB	DIR	NINDS
Others:	Jordan Grafman, Ph.D.	Chief	CNS	MNB	DIR	NINDS
	Daniel Ruchkin, Ph.D.	Elec. Engineer	U. of Maryland School of Medicine			
	Wolfgang Miltner, Ph.D.	Psychologist	U. of Tuebingen, Germany			

COOPERATING UNITS (if any)

University of Maryland School of Medicine, College Park, MD; University of Tuebingen, Germany

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Cognitive Neuroscience Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Event-related brain potentials (ERP) were used to study cognitive processes such as short- and long-term memory, spatial attention and visual search, mental rotation, mental arithmetic, and language comprehension. ERP studies of normal subjects were intended to reveal the brain mechanisms underlying cognition. Studies of patients with neurologic disorders were intended to allow us to characterize better patients' information processing deficits while providing information on the physiologic mechanisms underlying these cognitive processes. Data collection was completed in ERP studies of dementia Alzheimer's and Parkinson's diseases, HIV disease, and progressive supranuclear palsy (PSP). The results from the HIV and PSP studies indicate that, in the earliest stages of subcortical disease, processing at the cortical level is more affected than processing at the subcortical level (i.e., resembling a cortical dementia). This pattern reverses as the subcortical disease progresses. A follow-up study on the modality specificity of deficits in PSP patients has been completed. Studies of the mechanisms underlying and affecting attentional processes continue and data analysis is complete in two studies, one on how normal controls visually search a spatial array for items previously stored in short-term memory, and the other on the effects of fatigue on attention in patients with chronic fatigue syndrome (CFS) and normal controls. Studies with Dr. Daniel Ruchkin have demonstrated that memory rehearsal processes are marked by large negative slow waves and that different brain areas are invoked to perform verbal and spatial short-term memory rehearsal processes. Additional studies on the nature of short- and long-term memory deficits in amnestic patients have been completed and data analysis has begun. A study of multiple sclerosis patients revealed that they have a selective deficit in their short-term memory for verbal materials. Data analysis continues on studies of temporal lobectomy patients, Turner's patients, and the maturation of cognitive processes. Studies with Dr. Wolfgang Miltner have been aimed at providing additional data on the neural generator mechanisms underlying cognitive processes. Patient and control data have been used to validate the predictions of Johnson's model of the variables controlling P300 amplitude. These data revealed that, contrary to the widely accepted notion, the P300 is a component whose amplitude represents the simultaneous utilization of a number of cognitive processing "modules." During recognition memory in controls and patients, these modules appear to indicate the presence and functioning of different memory systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02038-21 MNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Combined Clinical, Viral and Immunological Studies of Neuromuscular Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M.C. Dalakas, M.D., Chief, MNB, DIR, NINDS

OTHER: Edward Cupler, M.D., Neurologist, NDS, MNB, DIR, NINDS
Elizabeth Sekul, M.D., Neurologist, NDS, MNB, DIR, NINDS
M. Monzon, Ph.D., Special Expert, NDS, MNB, DIR, NINDS
I. Illa, M.D., Neurologist, NDS, MNB, DIR, NINDS
D. Stein, M.D., Neurologist, NDS, MNB, DIR, NINDS
R. Quarles, Ph.D., Biochemist, DMN, DIR, NINDS
B. Sonies, Ph.D., Speech Pathologist CC, DIR, NINDS
M. Ropka, M.D., Director, IP, NR
M. Agboatwalla, M.D., Child Specialist, Karachi, Pakistan
A. McLaughlin, Ph.D., DRRP, OD

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Neuromuscular Diseases

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical and laboratory studies are conducted to determine etiology (infection, immunity and/or genetics) of chronic diseases of the neuromuscular system and design effective therapies. Current studies involve patients with polymyositis/dermatomyositis, post-polio syndrome, amyotrophic lateral sclerosis (ALS), demyelinating polyneuropathies, neuromuscular diseases associated with HIV infection, hypokalemic periodic paralysis and Duchenne muscular dystrophy.

The pathogenesis of post-polio syndrome is explored with a series of electrophysiologic, virologic, immunologic and histologic studies. The findings are compared with those seen in patients with acute paralytic poliomyelitis and other motor neuron diseases. Persistent or mutant poliovirus is sought in these patients' tissues using tissue cultures, PCR, and *in situ* hybridization. Because abnormal immunoregulation was found in some patients, a double-blind placebo-controlled trial using prednisone was conducted. The mechanism of post-polio fatigue, a common and disabling symptom in many patients, is under study. The spectrum of neuromuscular disorders associated with HIV infection has been studied and the role of the virus in the cause of neuropathy or myopathy is investigated with a variety of immunocytochemical studies, *in situ* hybridization and PCR. The antiretroviral drug AZT was found to cause an unique myopathy characterized by abnormal mitochondria as determined by various morphologic, molecular, biochemical and immunocytochemical studies. A longitudinal study of HIV-positive patients that develop myopathic symptoms while on AZT is conducted with serial muscle biopsies to assess factors associated with the development of myopathy. Because patients with AZT-myopathy have low muscle carnitine, a controlled clinical trial using oral L- carnitine is now conducted.

Randomized-controlled clinical trials are conducted with high-dose intravenous immunoglobulin in patients with polymyositis/dermatomyositis, chronic inflammatory and paraproteinemic demyelinating polyneuropathies, ALS and Duchenne muscular dystrophy. A controlled study using Dichlorophenamide, a carbonic anhydrase inhibitor, is also conducted in patients with hypokalemic periodic paralysis

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02531-12 MNB

PERIOD COVERED

October 1, 1990 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies in Neuromuscular and CNS Diseases and Their Experimental Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M.C. Dalakas, M.D.	Chief, NDS	MNB, DIR, NINDS
OTHERS:	M. Monzon, Ph.D.	Special Expert, NDS	MNB, DIR, NINDS
	I. Illa, M.D., Ph.D.	Visiting Associate, NDS	MNB, DIR, NINDS
	R. Quarles, Ph.D.	Biochemist	DMN, DIR, NINDS
	A.A. Ilyas, M.D.	Biochemist	N.J. Medical School
	N.D. Epstein, M.D.	Molecular Biologist	CHB, DIR, NHLBI

COOPERATING UNITS (if any)

LAB/BRANCH

Medical Neurology Branch, CNP, DIR

SECTION

Neuromuscular Diseases

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [X] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Section runs the Laboratory of Muscle Enzyme Histochemistry that processes up to 300 muscle and nerve biopsies per year for diagnostic studies. Examined muscle specimens are from patients with neuromuscular manifestations related to systemic autoimmune, viral, metabolic, endocrine or infectious diseases, and from patients with primary neuromuscular disorders, such as polymyositis, dermatomyositis, neurogenic muscular atrophies, muscular dystrophies, post-polio syndrome, mitochondrial encephalomyopathies, and biochemical or genetic muscle diseases such as central core disease or hypertrophic cardiomyopathy. The laboratory is also involved in the following immunologic, biochemic and virologic studies that examine the susceptibility of the muscle and nerve to immune or viral mediated injuries: (a) Study the regeneration of human muscle in health and disease and the maturation of satellite ceils by examining the expression of neural cell adhension molecules and laminins; (b) study the susceptibility of muscle and nerve to infection with retroviruses and the ability of HIV or HIV-infected lymphoid cells to infect human myotubes in culture and induce expression of MHC-antigens; (c) study the expression of the poliovirus receptor in human muscle and the ability of the poliovirus to infect and replicate in human myotubes; (d) study the effects of cytokines and lymphokines on human muscle myotubes and examine the role of ICAM-I in enhancing myocytotoxicity by promoting the adhesion of cytotoxic T cells to myotubes; (e) study the toxicity of AZT to muscle mitochondria by applying AZT to human muscle in culture; (f) study the effect of L-carnitine in reversing the mitochondrial abnormalities induced by AZT on myotubes and (g) use animal models to study: i) the pathogenesis of retrovirus- induced inflammatory myopathy by examining muscles from monkeys infected with the simian immunodeficiency virus; ii) the mechanism of AZT-induced mitochondrial myopathy by examining the structural, metabolic and functional alterations in the muscle mitochondria of healthy rats injected with AZT; and iii) the effect of L-carnitine in reversing or improving the AZT-induced myopathy in the rats.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02240-17 NEB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Epidemiology of Dementia and Other Neurodegenerative disorders

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Gustavo C. Roman, M.D. Chief NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Analytic studies to determine risk factors for vascular dementia (VAD) and Alzheimer's disease (AD) are planned or being conducted. International studies on the prevalence and incidence of dementia and Parkinson's disease are planned in Argentina, Brazil, Colombia, and Panama. Risk factors associated with local conditions will be determined.

These studies are in the planning stages.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02307-17 NEB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Educational Resources in Neurological Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Gustavo C. Roman, M.D. Chief NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because there is a severe shortage of available manpower in neuroepidemiology, the Neuroepidemiology Branch has developed an active teaching program for current and future collaborative investigators. Particular attention has been given to junior members of the American Academy of Neurology (Neurology residents). The NEB has participated actively in the Annual Courses of the American Academy of Neurology, in an effort to increase the interest in neuroepidemiology. To facilitate international research studies, educational activities have also been conducted in other countries.

The following are some of these activities:

Full-day neuroepidemiology course, American Academy of Neurology: *"Tools for Practice and Research: Understanding Neuroepidemiology," New York, NY.*
World Federation of Neurology, Research Group on Neuroepidemiology Annual Meeting, New York, N.Y.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02370-15 NEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Racial and Geographic Differences in Occurrence of Neurologic Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Gustavo C. Roman, M.D. Chief NEB, DIR, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.05	PROFESSIONAL: 0.05	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[X] (a1) Minors

[X] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of these studies is to accurately document possible racial, environmental and geographic differentials in the prevalence of major neurologic disorders by surveying an entire geographically defined population. The disorders investigated included cerebral palsy, dementia, psychomotor delay, epilepsy, Parkinson's disease, essential tremor, multiple sclerosis, and cerebrovascular disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 0271S-08 NEB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Epilepsy Neuroepidemiology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Karin B. Nelson, M.D.	Medical Officer	NEB, Dir, NINDS
Others:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS
	William Theodore, M.D.	Medical Officer	MNB, DIR, NINDS
	Sherrie Emoto, Ph.D.	Staff Fellow	BFSB, DIR, NINDS
	James Dambrosia, Ph.D.	Statistician	BFSB,DIR,NINDS

COOPERATING UNITS (if any)
Judith Manelis, M.D., Western Galilee Regional Hospital, Israel
Shi-Chuo Li, Beijing Neurosurgical Institute, PRC

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [X] (a1) Minors
- [X] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several studies on convulsive disorders are being planned and tested for feasibility, or are in progress. A protocol is in effect for a clinical study of the Lennox-Gastaut syndrome(LGS), a severe childhood epileptic encephalopathy with significant morbidity, characterized by uncontrolled seizures, mental retardation, and possible mental deterioration, to define the pathophysiology and anatomic locus of disturbance in LGS. We are evaluating the feasibility of performing randomized and placebo-controlled clinical trials of treatment after an initial convulsion in subjects presenting for care to a consortium of hospitals in Jerusalem.

With Yugoslav colleagues, we are examining the utility of the electroencephalogram as a predictor of recurrence of febrile seizures in a defined population in Yugoslavia.

This project has been completed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02746-07 NEB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Phenobarbital Clinical Trial in Children with Febrile Seizures*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
Others:	Deborah Hirtz, M.D.	Pediatric Neurologist	DNB, DCDND, NINDS
	Young Jack Lee, Ph.D	Mathematical Statistician	NEB, DIR, NINDS
	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS

COOPERATING UNITS (if any)
Jacqueline Farewell, M.D , Dept. of Neurosurgery, Univ. of Washington, Seattle, WA

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[X] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of the study are to assess the effects of phenobarbital, a commonly prescribed anti-convulsant, on tests of intelligence and behavior in children. The design of this study permitted comparison of measures of tested intelligence and of behavior in children with febrile seizures who had been treated with phenobarbital, and in a group of seizure-free control children. A comparison of the groups allowed assessment of benefit and risk of treatment for a common childhood neurologic problem.

*[This study supports the DNB/ND/NINDS contract study entitled: "Behavioral and cognitive side effects of phenobarbital used for prevention of febrile seizure recurrence." The project officer is Dr. Deborah G. Hirtz, DNB, DCDND, NINDS, and the contractor of the study is the University of Washington.]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02838-03 NEB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Retroviral Diseases of the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Gustavo C. Roman, M.D	Chief	NEB, DIR, NINDS
Other:	Aurora K. Pajeau, M.D	Clinical Associate	NEB,DIR,NINDS

COOPERATING UNITS (if any)
William A. Blattner, M.D., C. DCE, EEB, NCI; Clarence J. Gibbs Jr, Ph.D., DIR, CNSS, NINDS

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The Neuroepidemiology Branch completed the initial groundwork and planning stage of a registry of HTLV-I infections of the nervous system to obtain data on the magnitude of this problem. Case-control studies will be undertaken to determine risk factors for the development of HAM/TSP. Patient registry should also allow future therapeutic trials.

There is also interest on the study of HIV dementia

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02861-02 NEB

PERIOD COVERED
October 2, 1991 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Guillain-Barre Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Gustavo C. Roman, M.D.	Chief	NEB, DIR, NINDS

COOPERATING UNITS (if any)
Pan American Health Organization (PAHO); Peking Union Medical College (PUMC), Beijing China

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
[X] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study will determine the incidence of Guillain-Barre syndrome in Latin America, as part of the Pan American Health Organization 's program for poliomyelitis surveillance. Studies are also in the planning stages in the People's Republic of China

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02862-02 NEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurocysticercosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I. Gustavo C. Roman, M.D. Chief NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

Julio Sotelo, M.D., Research Division, Felipe Garcia Pedroza, M.D. Neuroepidemiologia, Mexican National Institute of Neurology and Neurosurgery; Marcelo Cruz, M.D. Ecuadorean Academy of

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Neuroepidemiology Branch, is conducting a study to define the prevalence of neurocysticercosis (NCC) in populations hyperendemic for taeniasis. As seizures and headaches have been correlated with NCC, the NEB is studying the prevalence of epilepsy and headache in Naulinco, Veracruz, Mexico, to determine the frequency of NCC. This study will determine the natural history of NCC in endemic regions of Mexico and Ecuador.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02863-02 NEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The California Cerebral Palsy Project

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D. Medical Officer NEB, DIR, NINDS

COOPERATING UNITS (if any)

Dr. Judith Grether; Dr. Susan Cummins; Birth Defects Monitoring Group, Department of Health Services, California; Health Officers Association of California

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[X] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has established a population-based registry of children with cerebral palsy (CP) in four San Francisco Bay Area counties. Elements completed or in progress include a) Study of demographic and medical characteristics related to the occurrence of cerebral palsy. A higher percentage of children with CP now than formerly were low in weight at birth, consistent with the increased survival of infants of low and very low birthweight. Older mothers, especially those of high parity, and mothers or fathers aged under 20 years, were at greater risk of producing a child with CP. The risk of CP was not related to when in pregnancy prenatal care began, nor to level of technology of the hospital of birth. b) Cerebral palsy in twins. Twin pregnancies produced a child with CP twelve times more often than singleton pregnancies. Much, but probably not all, of this risk was related to the tendency of twins to be low in birthweight. Twins of unlike-sex pairs, necessarily dizygotic, were not at lower risk than twins of like sex pairs. If one twin died in utero, the surviving co-twin was more than 100 times more likely than a singleton to have cerebral palsy. Twinning is increasing in developing countries, and is likely to contribute more children with CP. Paper submitted, a confirmation completed in another population, and results submitted for publication. c) Very low birthweight and risk for cerebral palsy. This study is examining infants born weighing under 1500 g, cases and controls, for factors that may contribute to risk of CP in very low birthweight children. A poster on this work has been accepted for the annual meeting of the Child Neurology Society in the fall, 1993. d) Dental markers. Among children with cerebral palsy whose anterior primary teeth could be examined, a third had developmental defects of enamel. These enamel hypoplasias were more often associated with low birthweight and prematurity, but even among infants with CP who were not premature or low in birthweight were associated with need for intensive care in the newborn period. Analyses are now underway comparing infants with CP and birthweight 2500 g or more whose teeth showed enamel hypoplasias dating to a month or more before birth, with children with CP or similar birthweight but normal teeth, and comparing these with normal controls of the same birthweight group, to evaluate the possibility that enamel defects may be clues to prenatal events that contribute to cerebral palsy. One paper on this work published, another is in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02866-02 NEB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Reliability of Diagnoses of First Seizures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	Joseph M. Scheller, M.D.	Special Expert	NEB, DIR, NINDS
Others:	E. Stanley Emery, M.D.	I.P.A. Expert	NEB, DIR, NINDS
	Robert Abel, Ph.D.	Staff Fellow	BFSB, DIR, NINDS
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*
Steven Weinstein, M.D., Neurology Department, Children's Hospital National Medical Center.
James Chamberlain, M.D. Neurology Department and Emergency Medical Trauma Center, CHNMC

LAB/BRANCH
Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)
[X] (a) Human subjects
[X] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We examined the consistency of diagnosis of a first seizure in children seeking care at a multispecialty urban teaching hospital, investigating whether the episode described was a first seizure, a nonfebrile seizure, whether it was symptomatic of an underlying illness, and how that seizure should best be descriptively classified. Among other information sought will be the source of the medical history, training of person in medical facility who records the history, length of time from episode to recording of history. At least two versions of the history were being recorded, and a sample audiorecorded; versions of the diagnostic impressions are being compared for consistency and for patterns of any differences observed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02867 -02 NEB

PERIOD COVERED

October 1, 1992 through September 30 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurologic Morbidity and Its Antecedents Within the NCPP Dataset

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Co-P.I.:	Jonas Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS
	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS

COOPERATING UNITS *(if any)*

BFSB, NINDS

LAB/BRANCH

SECTION

Neuroepidemiology Branch,

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

- [x] (a) Human subjects
  - [x] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Collaborative Perinatal Project of the NINDS data set continues to be an important resource for information relating maternal and pregnancy and perinatal factors with neurologic outcome in the newborn and child. Current projects employing this material involve the investigation of seizure disorders and motor disability in twins, and the fetal heart rate monitoring by intermittent auscultation as related to neonatal and later neurologic outcome. A project on growth in cerebral palsy (CP), before and after birth, is planned.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02891-01 NEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Multiple Births and Cerebral Palsy in Western Australia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Karin B. Nelson, M.D Medical Officer NEB, DIR, NINDS

COOPERATING UNITS (if any)

Beverly Petterson, M.D., Fiona Stanley, M.D., Linda Watrson, Western Australian Research Institute for Child Health, Princess Margaret Hospital for Children, Perth 6001, Western Australia

LAB/BRANCH

Neuroepidemiology Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[X] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A population-based study in Western Australia, identifying children with cerebral palsy and linking information on plurality ascertained through vital statistics of the Health Department of Western Australia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02892-01 NEB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The EEG as a Predictor in Febrile Seizures

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Karin B. Nelson, M.D.	Medical Officer	NEB, DIR, NINDS
	Sherrie Ehrogo, Ph.D.	Staff Fellow	BFSB, DIR, NINDS
Others:	Jonas H. Ellenberg, Ph.D.	Chief	BFSB, DIR, NINDS
	Deborah Hirtz, M.D.	Medical Officer	DNB, NINDS

COOPERATING UNITS (if any)

Nikola Sofijanov, M.D., Milutin Dukovski, M.D., Marija Katurek, M.D., Pediatric Clinic of the University of Skopje, Macedonia (Former Yugoslavia)

LAB/BRANCH

Neuroepidemiology Branch and Biometry and Field Studies Branch

SECTION

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [ ] (b) Human tissues [ ] (c) Neither

[x] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

From a population study in Macedonia, all children with febrile seizures were evaluated in one child neurology unit, histories, physical examinations, and electroencephalograms (EEGs) recorded and children followed for two years.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02315-16 NB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Positron Emission Tomography

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P I	Giovanni Di Chiro, M D	Chief	NB, CNP, DIR, NINDS
Others:	R.A Brooks, Ph D	Staff Physicist	NB, NINDS
	R. S Miletich, M.D., Ph D.	Sr. Clinical Invest.	NB, NINDS
	M J Fulham, M D	Visiting Associate	NB, NINDS
	R. Raman, M D	Sr Staff Fellow	NB, NINDS
	M Quarantelli M D	Special Volunteer	NB, NINDS *

COOPERATING UNITS (if any)

CNB, NINDS; DIR, NINDS, NM, CC; BEIP, NCRR; SNB, NINDS; NIDDK; Georgetown U.

LAB/BRANCH

Neuroimaging Branch, CNP, DIR

SECTIONS

Clinical Studies and Experimental PET

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [x] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Positron emission tomography (PET) is a nuclear medicine technique which allows us to obtain some anatomic data (e g , axial, coronal or sagittal images of the brain) as well as dynamic functional data (such as regional cerebral glucose consumption rate). The unique property of PET is that it provides ph[illegible]g c and pathophysiologic information not available with any other imaging procedure. Using a variety of radiopharmaceuticals as tracers, we have investigated with PET, brain tumors, movement disorders (Parkinson's disease, in particular), the dementias, narcolepsy and cerebral involvement in AIDS. New information has been gathered, both in the basic and in the clinical (patient management) areas

* Continued:

E H Oldfield, M D	Chief	SN, NINDS
C V Kufta, M D	Staff Physician	SN, NINDS
M Hallett, M.D	Clinical Director	CNP, NINDS
I J Kopin, M.D	Director	DIR, NINDS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02073-20 NB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nuclear Magnetic Resonance (Imaging and Spectroscopy)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Giovanni Di Chiro, M.D.	Chief	NB, CNP, DIR, NINDS
Others:	R.A. Brooks, Ph.D.	Staff Physicist	NB, NINDS
	J.R. Alger, Ph.D.	Research Biochemist	NB, NINDS
	A. Barnett, Ph.D.	Research Physicist	NB, NINDS
	M.J. Fulham, M.D.	Visiting Associate	NB, NINDS
	R.S. Miletich, M.D., Ph.D.	Sr. Clinical Invest.	NB, NINDS
	J. Vymazal, M.D.	CEEI Fellow	NCRR*

COOPERATING UNITS (if any)

In vivo NMR Research Center; Diagnostic Radiology Department; BEIP, NCRR; Albert Einstein College of Medicine, NY

LAB/BRANCH

Neuroimaging Branch

SECTIONS

Clinical Studies and MR Spectroscopy

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[x] (a1) Minors
[ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our NMR imaging research is developing along the following lines: a) NMR spectroscopy (proton) in patients with tumors, stroke, epilepsy and lipid storage diseases; b) diffusion-perfusion imaging in patients with stroke and brain tumors; c) comparing clinical MRI imaging results with those of PET; d) analysis of iron accumulation in the basal ganglia of normal primates of various ages as well as in parkinsonian (MPTP) animals; e) analysis of the signal intensity from critical areas (basal ganglia) in patients affected by a variety of movement disorders; f) assessment of pulsatile CSF flow and of the "mobile" (normal) and "fixed" (pathologic) spinal cord; g) diffusion-perfusion imaging plus proton MR spectroscopy in experimental cerebral ischemia in cats and rats; h) *in vitro* studies of ferritin's NMR properties

---

*Continued:

A. Righini, M.D.	Visiting Fellow	NB, NINDS
C. Pierpaoli, M.D.	Visiting Fellow	NB, NINDS
R. Raman, M.D.	Senior Staff Fellow	NB, NINDS
G. Tedeschi, M.D.	Special Volunteer	NB, NINDS
R. O. Brady, M.D.	Chief	DMNB, NINDS
N. Barton, M.D.,	Section Chief	DMNB, NINDS
C. Baumgarner, Ph.D.	Chemist	NB, NINDS
J.M. Hallenbeck, M.D.	Chief	SB, NINDS
T.J. De Graba, M.D.	Medical Researcher	SB, NINDS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02202-18 NIB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological Studies in Patients with Multiple Sclerosis and Other CNS Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
Others:	Mary E. Smith, M.D	Clinical Associate	NIB	DIR	NINDS
	Tanya Lehky, M.D.	Clinical Associate	NIB	DIR	NINDS
	Michael Racke, M.D.	Senior Clinical Investigator	NIB	DIR	NINDS
	Rhonda Voskuhl, M.D.	Clinical Associate	NIB	DIR	NINDS
	Steven Jacobson, Ph.D.	Section Chief	NIB	DIR	NINDS
	Clara Pelfrey	Special Volunteer	NIB	DIR	NINDS

COOPERATING UNITS *(if any)*

Leroy Hood, M.D., Dept. of Molecular Biotechnology, U. Washington; Roland Martin, M.D., Assist. Prof., Tubingen U., Germany, Steven Beall, M.D., Dept. of Neurology, U. of British Columbia

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.2	PROFESSIONAL: 1.9	OTHER: .3

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[X] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall goal of this project is to assess genetic and immunological factors that contribute to the pathogenesis of neurological disease. Particular attention is focused on multiple sclerosis (MS) since this disease is thought to have an immunopathological basis. Genetic and immunological factors are being examined in well characterized, sporatic patients and in affected or unaffected members of families with multiple affected members and in identical or nonidentical twins either concordant or discordant for MS. Several new multiplex families have been identified and used to examine the cellular immune response to myelin basic protein (MBP). A T-cell response to MBP is present in both affected and unaffected individuals and does not appear to differ in HLA restrction, peptide specificity or T-cell receptor usage between affected and unaffected individuals. Various forms of immunosuppressive therapy are being tested in the treatment of MS using MRI parameters as a means of measuring efficacy and a subset of patients show a significant improvement with treatment with immunosuppressive drugs such as cyclophosphamide or cyclosporine supporting the hypothesis that MS has an immunological basis.

Patients with other inflammatory diseases of the central nervous system are bing examined to assess disease mechanisms. HTLV-I associated myelopathy/tropical spastic paraplegia (HAM/TSP) is being evaluated since it may represent an example of viral-induced immunopathological disease. Serpositive patients either with or without clinical disease have been identified. Immunological studies indicate that disease correlates with the presence of HTLV-I cytotoxic T cells (CTL). New techniques for identification of the virus genome in blood and tissue are being developed. HTLV-II has been identified in one patient with a neurological disease identical to HAM/TSP indicating that retroviruses other than HTLV-I can cause neurological disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02204-18 NIB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunologic Mechanisms in Experimental Autoimmune Diseases of the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Henry F. McFarland, M.D	Acting Chief	NIB	DIR	NINDS
Others:	Michael R. Racke, M.D	Senior Clinical Investigator	NIB	DIR	NINDS
	Mary E. Smith, M.D	Clinical Associate	NIB	DIR	NINDS
	Paul Drew, Ph.D.	Senior Staff Fellow	NIB	DIR	NINDS
	Benjamin Segal, M.D	Clinical Associate	NIB	DIR	NINDS

COOPERATING UNITS (if any)

Cedric S. Raine, Ph.D., Prof., Albert Einstein U.; Maria Spatz, M.D., Sect. Chief, SB, DIR, NINDS; Richard McCarron, Ph.D., Special Expert, SB, DIR, NINDS; Keiko Ozato, Ph.D., Sect. Chief, LDMI, DIR, NICHHD

LAB BRANCH

Neuroimmunology, CNP

SECTION

Neurological Disease Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 3.6	PROFESSIONAL: 1.8	OTHER: 1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
☐ (a1) Minors
☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Current research is focused on a chronic-relapsing model of experimental allergic encephalomyelitis (EAE). This disease is produced by the transfer of lymphocytes sensitized against MBP to syngeneic mice. The neurologic dysfunction is characterized pathologically by inflammation and primary demyelination. The immunological mechanisms responsible for the initial episode and the chronic disease are being investigated. Because the migration of immune cells from the blood into the central nervous system occurs before clinical disease, interactions between endothelial cells (EC) which form the blood-brain barrier and immune cells are being studied *in vitro*. The function of various adhesion molecules in both adherence and lymphocyte signaling are being examined. The effects of various cytokines on lymphocyte/EC interactions and on clinical disease are being studied

Previous studies demonstrated that transforming growth factor-β1 (TGF-β1) temporarily reduced the clinical severity of EAE. Current studies show that long-term administration of the related compound, TGF-β2 reduces the severity of the clinical course of the disease and decreases the inflammation and demyelination associated with EAE. Immunohistochemical studies demonstrate TGF-β in the EAE lesion. T cells producing TGF-β in the lesion may contribute to the relapsing nature of disease in EAE. The effect of agents known to induce TGF-β are being studied in the EAE model. Retinoids are a class of molecules which can induce TGF-β and which have profound effects on cell growth and differentiation. Administration of retinoids results in an improvement of the clinical course of EAE. Also, retinoids increase IL-4 and decrease IL-2, gamma interferon and tumor necrosis factor gene expression in MBP-specific T cells. These results indicate that retinoids may alter cytokine production on encephalitogenic T cells. The influence of environmental factors on autoimmune disease is also being examined. Bacterial superantigens which can cause nonantigen-specific T-cell receptor activation can produce sufficient activation of MBP-specific T cells to allow induction of disease in some mouse strains.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD(S) REVIEWED

TITLE OF PROJECT

PRINCIPAL INVESTIGATOR

COOPERATING UNITS

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL STAFF YEARS

PROFESSIONAL

OTHER

CHECK APPROPRIATE BOX(ES)

- (A) NEW PROJECT
- (B) CONTINUATION OF PROJECT
- (C) TERMINATION
- (D) MINOR REVISION
- (E) MAJOR REVISION

SUMMARY OF WORK

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02603-10 NIB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Lymphoid Cell-Cell Interactions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	William E. Biddison, Ph.D.	Section Chief	NIB	DIR	NINDS
Others:	Ursula Utz, Ph.D	Visiting Fellow	NIB	DIR	NINDS
	Tomiko Tsuchida, M D., Ph.D.	Visiting Fellow	NIB	DIR	NINDS

COOPERATING UNITS (if any)

John E. Coligan, Ph.D., Chief, Biological Resources Branch, DIR, NIAID; Hans J. Zweerink, Ph.D., Senior Scientist, Merck Research Labs, Rahway, N.J.

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Molecular Immunology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.9	PROFESSIONAL: 3.9	OTHER: 2.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general objective of this project is to define the mechanisms by which human lymphoid cells interact with antigen-presenting cells in order to produce and regulate immune responses. Over the past year, there have been three major efforts underway that are targeted on this objective: 1) analysis of expressed T-cell repertoires in multiple sclerosis (MS) patients and the contribution of T-cell receptor (TCR) germ-line genes to susceptibility to MS; 2) dissection of the molecular basis of viral peptide-binding and presentation for T-cell recognition by HLA class I molecules; and 3) analysis of antigen presentation pathways for class I-restricted antiviral cytotoxic T- lymphocyte (CTL) responses The principle findings are as follows: 1) analysis of T-cell responses to MBP and a foreign antigen (tetanus toxoid) by genetically identical twins who are concordant or discordant for MS indicates that there is a skewing of the TCR repertoire that correlates with the presence of MS; 2) an extension of previous studies has further localized a susceptibility gene(s) for MS to a 175-kb region of the TCR Vβ chain locus, and has demonstrated gene complementation between this susceptibility gene(s) and an HLA class II gene; and 3) isolation and sequencing of endogenous peptides bound to the HLA class I molecule HLA-A3 has permitted identification of a specific combination of peptide anchor residues which can be used to successfully predict immunogenic T-cell epitopes within viral protein sequences.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02817-04 NIB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Involvement of Human Retrovirus Associated with Chronic Neurologic Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Steven Jacobson, Ph.D.	Section Chief	NIB	DIR	NINDS
Others:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
	Irina Elovaara, M.D.	Visiting Fellow	NIB	DIR	NINDS
	Tanya Lehky, M.D.	Clinical Associate	NIB	DIR	NINDS
	Allan Kermode, M.D.	Special Volunteer	NIB	DIR	NINDS

COOPERATING UNITS *(if any)*

William Blattner, M.D., Chief, VES, NCI; Bernard Poiesz, M.D., Chief, Dept. of Med. and Microbiology, SUNY Health Sci. Center; Thomas Waldmann, Chief, MET Branch, NCI; Anthony Fauci, M.D. Dir. NIAID *

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Neurological Disease Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The general goals of this project is to define 1) the role of human retroviruses that are associated with chronic-progressive neurologic disease and 2) the host immune responses to these agents that may be involved in the pathogenesis of these disorders. Over the past year, a number of specific research efforts have been targeted to address these broad issues. They include: 1) the continued definition of $CD8^+$, HTLV-I specific, HLA class-I-restricted cytotoxic T lymphocyte (CTL) from patients with HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and the role these cells play in the pathogenesis of this disorder; 2) the detection of HTLV-I in the central nervous system of HAM/TSP patients by use of in situ hybridization techniques and the development of in situ-polymerase chain reaction to amplify low viral copy number in these tissues; 3) the demonstration of HTLV-I-specific T-cell responses from HTLV-I seronegative individuals at risk for exposure to HTLV-I and; 4) the molecular characterization of human retroviruses isolated from patients with HAM/TSP and other chronic-progressive neurologic diseases. The major findings of these studies are; 1) $CD8^+$, CTL directly isolated from peripheral blood lymphocytes or cerebrospinal fluid of HAM/TSP patients are specific for immunodominant peptides of the tax region of HTLV-I and are restricted to particular HLA alleles; 2) exceptionally high precursor frequencies were demonstrated to these peptides; 3) immunodominant peptides can be used to anergize or delete these cells; 4) HTLV-I tax mRNA signals were detected in spinal cord lesions of HAM/TSP patients; 5) the technique of *in situ*-PCR was developed and successfully amplified HTLV-I tax DNA from PBL of HAM/TSP patients; 6) HTLV-I responses to synthetic peptides of HTLV-I could be demonstrated from HTLV-I seronegative, PCR negative individuals known to be exposed to this virus; 7) HTLV-I molecular sequences were identified in an HTLV-I seronegative individual with a chronic-progressive neurologic disease; 8) HTLV-II has been unequivocally identified, both molecularly and immunologically, in an individual with a chronic myelopathy indistinguishable from HAM/TSP.

*Continued: Cedric S. Raine, Ph.D., Professor, Albert Einstein U.
Scott Koenig, M.D., Head, Immunology, MedImmune, Inc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02831-03 NIB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Class II Major Histocompatibility Complex Genes in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Elliot P. Cowan, Ph.D	Special Expert	NIB	DIR	NINDS
Others:	Steven Jacobson, Ph.D	Section Chief	NIB	DIR	NINDS
	Tanya J. Lehky, M.D	Clinical Associate	NIB	DIR	NINDS
	William E. Biddison, Ph.D.	Section Chief	NIB	DIR	NINDS
	Tomiko Tsuchida, M.D., Ph.D	Visiting Fellow	NIB	DIR	NINDS

COOPERATING UNITS *(if any)*

Lois A. Lampson, Ph.D., Associate Professor, Department of Neurology, Harvard Medical School

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.6	PROFESSIONAL: 1.5	OTHER: 1.1

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Accumulated evidence points to cytotoxic T-lymphocyte (CTL) recognition of HTLV-I-infected neuronal cells as a pathogenic mechanism in HAM/TSP. However, neuronal cells normally do not express detectable levels of class I or class II MHC molecules, which are required for presentation of antigen to T cells. In an effort to resolve this paradox, we have demonstrated that HTLV-I infection of human neuroblastoma cell lines (HNCL), as a model of neuronal cells, resulted in the expression of HLA-class I and class II (DR, DP, and DQ) molecules, and that class II expression was dependent upon HTLV-I infection. Studies over the past year have further characterized HTLV-I-induced class II expression on the HNCL at the cellular and molecular levels. The major findings are as follows: 1) HTLV-I-infected neuroblastomas expressing HLA-class II molecules can be recognized by superantigen-specific, class II-dependent CTL lines, demonstrating that class II is functionally expressed on the HNCL; 2) binding of proteins to the X2 box of the HLA-DRA promoter correlates with class II expression in the HTLV-I-infected HNCL, and may represent a complex of transacting factors induced by infection that results in class II expression; 3) stable transfectants of HNCL have been generated using a construct that contains the HTLV-I Tax gene under the control of the metallothionein promoter, allowing controlled expression of Tax. Expression of Tax in the HNCL results in HLA-DRA expression, but not class I expression, demonstrating that a viral gene is, at least in part, responsible for HLA-class II induction in the HNCL.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02853-02 NIB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Examination of Natural History and Therapy of Multiple Sclerosis Using MRI

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Henry F. McFarland, M.D.	Acting Chief	NIB	DIR	NINDS
Others:	Lael Stone, M.D.	Special Volunteer	NIB	DIR	NINDS
	Suhayl Dhib-Jalbut, M.D.	Special Volunteer	NIB	DIR	NINDS
	Michael K. Racke, M.D.	Clinical Associate	NIB	DIR	NINDS
	Tanya J. Lehky, M.D.	Clinical Associate	NIB	DIR	NINDS

COOPERATING UNITS (if any)

Joseph A. Frank, M.D., Director, LDDR; Paul Albert, Ph.D., BFS, DIR, NINDS; Mark Armstrong, M.D., DRD, CC, NIH

LAB/BRANCH

Neuroimmunology, CNP

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.8	PROFESSIONAL: 1.8	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

[X] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of this project is to use magnetic resonance imaging (MRI) to examine the natural history and potential therapeutic approaches in multiple sclerosis (MS). Emphasis has been placed on investigation of the early MS lesion which is characterized by enhancement on T1 weighted MRI images following administration of gadolinium-DTPA (Gd). Results from initial studies have indicated that MS can be an active disease, even during periods of remission in the early relapsing-remitting phase of the illness. The correlation between the frequency or area of Gd-enhancing lesions and episodes of clinical worsening has been examined. Although most Gd-enhancing lesions occurring in the cerebrum are clinically silent, episodes of worsening tend to occur during periods of increased disease activity as evidenced by increased frequency or area of enhancing lesions. The clinical symptoms and signs generally are due to lesions occurring in the spinal cord or brain stem concurrently with the increased activity in the cerebrum. These results demonstrate a correlation between clinical worsening and periods of increased disease activity occurring in the cerebrum and indicate that the regulation of disease activity as measured by Gd enhancement seems similar in the cerebrum and spinal cord.

Examination of the pathological changes occurring in conjunction with Gd enhancement indicate an acute inflammatory process with prominent perivascular cuffs of lymphocytes. These findings support the hypothesis that Gd enhancement represent the initial step in lesion development. Treatment trials using MRI as the primary outcome measure are now underway and indicate that the response to therapy is heterogeneous further complicating assessment of the results of clinical trials. This later finding strengthens the need for an objective measure of disease activity, such as MRI, to assess results of therapeutic trials.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 NS 02886-01 SB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Quantification of Neurologic Deficit Progression in Acute Stroke Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I:	B Kelly, M D	CMD, USN	Neurology, NNMC
Others	T DeGraba, M.D	Senior Staff Fellow	SB, NINDS
	J Hallenbeck, M D	Chief	SB, NINDS
	P Oberlander, R N	Patient Coordinator	SB, NINDS

COOPERATING UNITS (if any)
A Dutka, M D , Department of Neurology, National Naval Medical Center

LAB/BRANCH
Stroke Branch

SECTION
Clinical Investigation Section

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0 9	0 55	0 40

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[x] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stroke has been traditionally regarded as a catastrophic event in which maximal damage to brain tissue occurs almost immediately. Recently, clinical and animal research has revealed that the ultimate degree of tissue damage in a stroke is not determined in the first few minutes but instead evolves over a period of hours to days. Amplification of excitotoxic neurotransmitter release, progressive intracellular calcium accumulation, blood-brain barrier compromise, and regional inflammation all may play a role in delayed neuronal death. In conjunction with monitoring physiologic variables, including blood pressure and oxygen saturation, careful observation of clinical neurologic progression may provide an understanding of the "window of opportunity" for acute interventional therapy. The primary objective of this study is to monitor all consecutive stroke patients admitted to the National Naval Medical Center (NNMC) within 24 hr of the onset of cerebral ischemic symptoms and record the progression of clinical deficits over the first 48 hr. A standardized examination (the NIH Stroke Scale) will be performed on admission and again every 8 hr for 48 hr. The patients will be monitored in the neurology ICU and a continuous recording of blood pressure, heart rate, and oxygen saturation will be obtained over this same period. The goal of analysis is to identify the percent of NNMC patients who develop significant progression in the acute post-ischemic period. If substantive progression (30-40%) is found in this population of patients, further investigative and interventional studies are warranted to understand and treat early stroke progression.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02887-01 SB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Activation of Cytokines, Leukocytes, and Endothelium After Acute Cerebral Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P I:	T DeGraba, M D	Senior Staff Fellow	SB, NINDS
Others:	J. Hallenbeck, M D	Chief	SB, NINDS
	P. Oberlander, RN	Patient Corrdinator	SB, NINDS
	R McCarron, Ph.D	Special Expert	SB, NINDS
	M Spatz, M.D	Section Chief	SB, NINDS

COOPERATING UNITS (if any)

B. Kelly, M.D., Dept. of Neurology NNMC, V. Aletich, M.D., Dept. of Radiology, M Foust, M D , N Bakalar, M D , T Porter, M D , Dept of Psychiatry, NNMC

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS	PROFESSIONAL:	OTHER:
0 75	0 45	0.30

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[x] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Effective management of acute stroke patients has remained elusive to the present date Recent evidence indicates that increased cytokine levels and leukocyte and endothelial cell activation may play a major role in secondary neuronal injury after acute focal cerebral ischemia The purpose of this investigation is to more clearly characterize the role of the inflammatory response after ischemic injury in humans with regard to its causal influence on secondary neuronal injury and predictive value for long-term functional outcome. Blood samples will be drawn from acute stroke patients on admission and serially for the first 7 days Serial neurologic exams will also be performed and MRI scan of the head will be done to determine infarct size Depression scales will be done within the first 14 days All patients will be seen at 90 days after the ischemic event for follow up at which time blood cytokine levels, neurologic outcome scales, and depression scales will be performed Through analysis of the advent and duration of cytokine activation, we hope to establish a correlative relationship between the postischemic inflammatory response and neuronal injury Given the published work demonstrating neuronal protection after ischemia in animal models with antagonists of leukocyte activation or of the inflammatory pathways, we expect these results to establish a temporal window for future drug trials in reducing infarct size after acute stroke. In addition, since clinical outcome in stroke is also dependent on rehabilitation effort, the incidence of depression in stroke patients becomes an important variable in long-term outcome Thus, we will observe the incidence of depression in stroke patients as it relates to the volume and location of cerebral infarction A novel approach of correlating sleep architecture in stroke patients with the incidence of mood disturbance will be performed by obtaining a polysomnogram in patients 3-6 months after the ischemic event A comparison will be made between patients with and without depression Polysomnograms will also be compared against those of patients with primary depression (who display a very characteristic sleep pattern). It is hypothesized that the mood disturbance in stroke patients may actually be a result of altered sleep patterns caused by the neuronal injury This may lead to a new understanding of the etiology of mood disorders in stroke patients and aid in their treatment

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 NS 02888-01 SB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytokine, Leukocyte and Endothelium Activation in Risk Factors for Stroke

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T DeGraba, M D	Senior Staff	SB, NINDS
Others:	J Hallenbeck, M D	Chief	SB, NINDS
	P Oberlander, RN	Patient Coordinator	SB, NINDS
	R. McCarron, Ph.D.	Special Expert	SB, NINDS
	M. Spatz, M D	Section Chief	SB, NINDS

COOPERATING UNITS (if any)

B. Kelly, M.D., A Dutka, M.D., Dept. of Neurology, NNMC, V. Aletich, M.D., Dept of Radiology NNMC; C. Cunningham, M.D., Dept. of Vascular Surgery, R. Hargraves, M D., Dept. of Neurosurgery, NNMC

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.75	0 45	0 30

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[x] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major risk factors which are associated with an increased incidence of stroke have been known for many years. However, the basic mechanisms by which these factors lead to the increased risk are not fully understood Preliminary studies indicate that activation of the immune system by risk factors for stroke (hypertension, hypercholesterolemia, diabetes and age) increases the risk of endothelial activation and the formation of intravascular thrombosis By measuring the levels of cytokine and monocyte, macrophage and endothelial cell activation in the stroke-prone population and age matched controls without risk factors, an attempt will be made to characterize those factors which potentially increase the risk for activation of brain vessel endothelium as well as preparing the brain tissue (including the cerebral vasculature) for a hyperactive inflammatory response to an ischemic insult In addition, although disease is a major cause of stroke in the U.S., no radiographic findings related to the stenosis nor specific morphologic features of the atherosclerotic plaque have been useful in predicting which will become symptomatic and which will remain asymptomatic. This study will analyze carotid endarterectomy surgical specimens from symptomatic and asymptomatic patients for leukocyte adhesion molecules on the plaque endothelial cells using immunofluorescence staining Blood drawn at the time of preoperative testing will be examined for leukocyte and endothelial cell activation by fluorescence activated cell sorting (FACS) and baseline cytokine levels It is hypothesized that the local release of cytokines and the expression of endothelial cell surface leukocyte receptors play a major role in the conversion of an asymptomatic plaque to a symptomatic one Understanding the role of cytokines, leukocyte activation, and endothelial interaction in promoting the cerebral ischemic state may lead to a novel approach in future stroke prevention regimens

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02885-01 SB

PERIOD COVERED

December 20, 1992 through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Gene Activity in Astrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.	Michael Brenner, Ph.D.	Special Expert	SB, NINDS
Others:	Qi Zhang, Ph.D.	Visiting Fellow	SB, NINDS

COOPERATING UNITS *(if any)*

A.H. Koeppen, MD (VAMC, Albany, NY); K. Liu, MD, D.L. Yao, MD (LEP, NINDS, NIH); A. Messing, Sch. Vet. Med., Univ. Wis., Madison, WI); J. Schwartz, PhD (CNB, NINDS); S.K. Shore, PhD, CRCB, NCI); E. Browning, PhD, Robt. Wood Johnson Med. Sch., Piscataway, NJ)

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
0.6	0.6	0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Astrocytes play important roles in the development, maintenance and response to injury of the CNS. To understand and manipulate astrocyte function, this project addresses transcriptional control of the GFAP gene, which encodes the astrocyte-specific intermediate filament protein glial fibrillary acidic protein (GFAP).

Using transfection of astrocytoma cells with reporter gene constructs, multiple segments within the GFAP basal promoter and upstream region have been identified that interact to control expression of the gene. Site-directed mutagenesis is being used to pinpoint the critical specific sequences within these segments. This will be followed by isolating and studying the regulatory proteins acting at these sites.

The activity of reporter constructs is also being examined in transgenic mice. A 2,000 base pair 5-flanking fragment of the GFAP gene has been found sufficient to drive expression of a β-galactosidase reporter gene in astrocytes throughout the CNS. Deleting from this construct a GFAP segment found unimportant for expression in cultured cells also produces expression exclusively in astrocytes, but activity is largely restricted to the cortex. These results indicate that astrocytes are heterogeneous in gene expression and that different regulatory regions of the GFAP gene are utilized by different types of astrocytes. Projects are also underway, to use the GFAP regulatory sequence to express other genes of interest in astrocytes to study brain development and function and to produce models for human diseases. Genes currently being expressed include those encoding the amyloid precursor protein associated with Alzheimer's disease, somatostatin, TGF-β1, herpes simplex virus thymidylate kinase (HSV-TK), and a putative dominant negative GFAP.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02856-02 SB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hibernation - A New Approach to Stroke Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	J. M. Hallenbeck, M.D.	Chief	SB, NINDS
Others:	K. U. Frerichs, M.D.	Visiting Fellow	SB, NINDS
	M. Brenner, Ph.D.	Special Expert	SB, NINDS

COOPERATING UNITS (if any)

L. Sokoloff, MD, C. Kennedy, LCM.NIMH; J. Joy, MD and C. Merril, MD, NIMH; H. Gainer, Ph.D., H. Jaffe, Ph.D, LNC/NINCDS

LAB/BRANCH

Stroke Branch

SECTION

Clinical Investigation Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.47	1.47	1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Efforts to develop effective measures for the treatment of stroke have generally been based on the implicit assumption that one, or at the most several, factors control the progressive brain injury that occurs during the early hours of focal brain ischemia. Postischemic progression of brain damage appears to be extremely multifactorial. There is a finite probability that the assumption underlying most therapeutic stroke trials that seek to identify a dominant or controlling factor that determines postischemic progression of brain damage is incompatible with the fundamental nature of the problem. Postischemic progression of brain damage may be the result of a constellation of minor causes and the quest for a dominant or controlling cause would then be ultimately futile.

This project continues to investigate mammalian hibernation, a state of natural tolerance to severely reduced blood flow and oxygen delivery. Efforts to isolate and identify the factor or factors that regulate the controlled metabolic depression that forms the essence of natural hibernation are in progress. Such factors with pleiotropic effects may have benefit in the treatment of progressive brain damage in human stroke that is characterized by loss of homeostatic control due to activation of a multitude of pathophysiological postischemic events. The existence of regulatory factors in hibernation is supported by several findings that render passive submission to the effect of ambient temperature unlikely: (1) The onset and rate of development of bradycardia and reduced oxygen consumption during the transition to hibernation is rapid and precedes a more gradual drop in body temperature. (2) Regulation of enzyme function and gene expression that contributes to preservation of homeostasis during hibernation has been demonstrated. (3) Artificially induced hypothermia leads to rapid death in animals otherwise able to tolerate the same degree of hypothermia during natural hibernation. The identification of these putative control mechanisms may enable us to prevent or minimize the breakdown of homeostasis and cellular damage in cerebral ischemia in other species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02801-05 SB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions Between Cerebrovascular Endothelial Cells and Immune Leukocytes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	R. M. McCarron, Ph.D.	Special Expert	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been subsumed within project Z01 NS 02865-02 SB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
ZO1 NS 02802-05 SB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immune Mechanisms: Regulation of EC Surface Antigen Expression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I:	R.M McCarron, Ph D	Special Expert	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH
Stroke Branch

SECTION
Section of Neurocytobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been subsumed within project Z01 NS 02865-02 SB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02324-17 SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Blood-Brain Barrier: *In Vitro* Model for the Study of Cerebrovascular Endothelial Permeability

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R.M. McCarron, Ph.D.	Special Expert	SB, NINDS
Others:	M. Spatz, M.D.	Section Chief	SB, NINDS
	H. Ishii, M.D.	Visiting Fellow	SB, NINDS
	A. Strasser, D.V.M.	Guest Researcher	SB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.2	OTHER: 0

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
  [ ] (a1) Minors
  [ ] (a2) Interviews

[ ] (b) Human tissues

[x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The experiments performed here are currently based on the hypothesis that oxygen radicals generated as a result of central nervous systems (CNS) injury and/or schemia contribute to pathogenesis, at least in part, by altering endothelial membrane integrity. Due to the limited availability of cultured cerebrovascular endothelial cells (EC) which constitute the blood-brain barrier (BBB), only a minimal number of experiments were able to be performed under this project title. However, these experiments demonstrated that oxygen free radicals such as superoxide anion (O_2^-) and hydroxyl (OH^-) generated by EC may be responsible for alterations in BBB permeability known to occur in neuropathologic disorders such as stroke.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02865-02 SB

PERIOD COVERED
October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Interactions Between Cerebrovascular Endothelial Cells and Blood Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator that participate in the project. Indicate their titles, laboratory and institute affiliation)

P.I.:	R.M. McCarron, Ph.D.	Special Expert	SB, NINDS
Others:	J.M. Hallenbeck, M.D.	Chief	SB, NINDS
	M. Spatz, M.D.	Section Head	SB, NINDS
	L. Wang, M.D.	Guest Researcher	SB, NINDS

COOPERATING UNITS (if any)
A-L. Siren, L. Yong, Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD

LAB/BRANCH
Stroke Branch

SECTION
Section of Neurocytobiology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS: 3.5	PROFESSIONAL: 2.1	OTHER: 1.4

CHECK APPROPRIATE BOX(ES)
- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [X] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

These experiments investigate interactions between cerebrovascular endothelial cells (EC) which constitute the blood-brain barrier (BBB) and peripheral blood cells and/or components. Adhesive interactions involving murine cerebrovascular EC and encephalitogenic T lymphocytes were up regulated by the cytokines tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interferon-γ (IFNγ), and inhibited by transforming growth factor-β (TGFβ). Cerebrovascular EC lines were also derived from both spontaneously hypertensive (SHR) rats and normotensive Wistar-Kyoto (WKY) rats. Although both cell lines constitutively expressed similar levels of the adhesion molecule, intercellular adhesion molecule 1 (ICAM-1), SHR-derived EC were more sensitive than WKY-derived EC with regard to ICAM-1 up-regulation induced by suboptimal concentrations of TNFα, IL-1β and IFNγ, as well as by lipopolysaccharide (LPS). Although both cell lines demonstrated similar maximal response at high cytokine concentrations, the level of ICAM-1 up regulation to all concentrations of LPS was significantly greater in SHR derived EC. Additional experiments examining the adhesion of syngeneic as well as allogeneic monocytes indicate similar increased responsiveness of cytokine- or LPS-treated EC derived from SHR rats vs WKY rats. These results indicate a mechanism by which hypertension may be a predisposing factor to disorders (i.e., stroke) related to increased adhesive interactions between monocytes and endothelium. Experiments were also performed on EC derived from human brain. In addition to the aforementioned factors, it was observed that endothelin (ET-1, ET-2 and ET-3), a family of potent vasoconstrictive peptides, up regulated ICAM-1 and VCAM-1 and induced the expression of E-selectin on these cells. Studies regarding ET-1 induced release of ^{51}Cr from EC demonstrated alterations in endothelial "permeability" coincident and proportional to effects seen on adhesion molecule expression, indicating additional functional parameters affected by this peptide. All the above findings implicate factors such as cytokines and vasoactive peptides in disorders involving recruitment, attachment and/or transvascular migration of blood cells at sites of inflammatory responses. The data demonstrate the enhanced release of proinflammatory factors such as TNF, and responsiveness (i.e., ICAM-1 expression or monocyte adhesion) to proinflammatory factors in aged and hypertensive rats, respectively. Such findings may explain how advanced age and hypertension act as risk factors for stroke (i.e., increase the likelihood of interactions between monocytes and endothelium leading to local thrombosis or hemorrhage).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02776-05 SB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Production of Experimental Allergic Encephalomyelitis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	R.M. McCarron, Ph.D	Special Expert	SB, NINDS
Others:	L. Wang, M.D.	Visiting Fellow	NIB, NINDS

COOPERATING UNITS (if any)

Dr. M.K. Racke, NIB, NINDS

Dr. R.S. Fujanami, Dept. Neurol., Univ. of Utah, Salt-Lake City, UT

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.2	0.3

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiments were conducted to discern differences between encephalitogenic and non-encephalitogenic myelin basic protein (MBP)-responsive helper T cells. These cells comprise cell lines which were demonstrated to passively transfer experimental allergic encephalomyelitis (EAE) in naive mice. Cells from an encephalitogenic T cell line were plated atop monolayers consisting of syngeneic cerebrovascular endothelial cells (EC) which were untreated or treated with tumor necrosis factor-α (TNFα). Nonadherent T cells removed from the TNFα-treated EC culture monolayer failed to transfer EAE, whereas, those nonadherent to untreated EC culture monolayers exhibited enhanced ability to passively transfer EAE. These findings suggest that discrimination between EAE-inducing and non-encephalitogenic MBP-reactive helper T cells occurs at the level of adhesion to EC which constitute the blood-brain barrier (BBB). These findings have important implications regarding pathogenic mechanisms of adoptively transferred EAE and the role of adhesion to cerebrovascular EC in this animal model for the human autoimmune disorder, multiple sclerosis.

Experiments were also conducted to characterize the infectibility and role (i.e., antigen-presenting function) of cerebrovascular EC in immune responses to murine viruses (i.e., Theiler's murine encephalomyelitis virus and vaccinia virus). The data indicated that although EC cultures did not function as targets for cytolysis by Theiler's murine encephalomyelitis virus-immune spleen cells, they were able to do so for cytotoxic T cells from vaccinia virus-immune mice. Similar findings were obtained with regard to the capacity of EC to function as antigen-presenting cells. The data indicate cerebrovascular EC cultures are a valuable resource for the study of biology and immune response to murine viruses, such as Theiler's virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02689-09 SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Endothelin and Prostanoid Production in Cerebromicrovascular Endothelium

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	M. Spatz, M.D.	Section Chief	SB, NINDS
Others:	D. Stanimirovic, M.D., Ph.D.	Visiting Fellow	SB, NINDS
	R.M. McCarron, Ph.D.	Special Expert	SB, NINDS

COOPERATING UNITS (if any)

S. Uematsu, M.D., Johns Hopkins Hospital, Baltimore, MD

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.67	0.35	0.32

CHECK APPROPRIATE BOX(ES)

[ ] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Vasoconstrictive peptides and prostanoids have been implicated in the pathogenesis of hypertension and vasospasm. Recently, we have shown that vasopressin (AVP) or angiotensin II (Ang II) stimulates receptor-mediated production of ET-1 and/or PGD_2 in endothelium derived from capillaries and microvessels of human brain. These studies indicate that the same specific AVP or Ang II receptors can mediate both the formation of a vasoconstrictor ET-1 and vasodilator prostanoid PGD_2. In view of these observations, we investigated the temporal dynamics and cellular mechanisms of ET-1 and prostanoid production induced by either AVP or Ang II in human brain endothelial cells (HBEC).

This study demonstrates that both AVP and Ang II stimulated secretion of both immunoreactive ET-1 and prostanoids from HBEC by a receptor-mediated induction of phospholipase C (PLC) and phospholipase A_2 (PLA_2). The highest level of constitutive or AVP- or Ang II-induced ET-1 was observed after 24 hr incubation of HBEC. The temporal profile of AVP-stimulated production of prostanoics differed from that of Ang II. AVP-induced accumulation of prostaglandin D_2 (PGD_2) persisted for 24 hr while Ang II-stimulated PGD_2 was only seen at 4 hr. Ang II stimulated PGI_2 secretion maximally at 8 hr, whereas AVP did not stimulate PGI_2 secretion. Dexamethasone (Dxm), indomethacin (Indo), and nordihydroguiaretic acid (NDGA), the respective inhibitors of PLA_2/cyclooxygenase II, cyclooxygenase and lipoxygenase, increased both constitutive and AVP- or Ang II-stimulated secretion of ET-1. Dxm also decreased AVP- or Ang II-stimulated production of PGD_2 and prostaglandin $F_{2\alpha}$. The concomitantly observed stimulation of ET-1 and inhibition of prostanoid secretion by either Dxm or NDGA strongly suggest an intercommunication between these events which may play a role in regulating cerebral microvessel function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02357-16 SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia: Neurotransmitters, Metabolism and Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	Danica Stanimirovic, M.D., Ph.D.	Visiting Fellow	SB, NINDS
Others:	Maria Spatz, M.D.	Section Chief	SB, NINDS

COOPERATING UNITS (if any)

B. Mrsulja, Inst. of Biochemistry, Fac. Med., Belgrade, Yugoslavia

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, N.I.H., Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
0.45	0.35	0.10

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Numerous metabolic reactions (i.e., energy depletion, disturbed calcium homeostasis, acidosis, phospholipid degradation, rapid release of excitatory neurotransmitters, and accumulation of reduced substrates) triggered by brain ischemia, have been thought to play a role in the pathomechanism of brain injury. Although many of these reactions are initiated during ischemia alone, substantial brain damage develops during the reperfusion in models of transient ischemia.

In this study, we evaluated the effects of the following drugs: nimodipine (1 mg/kg b.w., i.p.), 2-amino-5-phosphonovaleric acid (4 mg/kg b.w., i.p.) and propentofylline (25 mg/kg b.w., i.p.), administered (alone or in combination) at the end of 15 min bilateral ischemia in gerbils on mitochondrial superoxide dismutase (SOD), glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PD), monoamine oxidase (MAO) activities, and thiobarbituric acid reactive material (TBARM) and brain water content at 1 hr of reperfusion. The combined treatment virtually abolished early postischemic brain edema (4.1% vs. 0.6%) and efficiently counteracted ischemia-inouced changes [decreased SOD (79% vs. 98%), GR (52% vs. 105%) and MAO (25% vs. 79%) and increased TBARM (198% vs. 108%)]. The same combination of drugs administered 15 min before ischemia had a similar effect (e.g., reduced brain swelling and lipid peroxidation) as when given at the end of ischemia, whereas a limited or absent impact was seen when the drugs were given 15 min or 1 hr after ischemia, respectively. The data suggest that free radical-mediated mitochondrial dysfunction and (post) schemia brain swelling can be reduced by drugs which synchronously prevent processes induced in the early stages of reperfusion.

These findings may provide a foundation for a novel approach to the treatment of free radical-mediated brain damage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02623-09 SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cerebral Ischemia and Edema: Biogenic Amines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M Spatz, M D	Section Chief	SB, NINDS
Others:	A. Strasser, D.V.M.	Guest Researcher	SB, NINDS
	H. Ishii, M.D	Visiting Fellow	SB, NINDSD.
	D Stanimirovic, M.D., Ph.D	Visiting Fellow	SB, NINDS

COOPERATING UNITS (if any)

B. Mrsulja, Inst. of Biochemistry, Fac. Med., Belgrade, Yugoslavia

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.95	PROFESSIONAL: 0 85	OTHER: 0.10

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The association of changes in the metabolic pathway of monoamines (dopamine and 5-hydroxytryptamine) with mitochondrial enzyme systems which are involved in the production and removal of free radicals formed during dopamine metabolism and formation of edema was investigated in bilateral brain ischemia in gerbils. The results suggest that the involvement of dopamine-derived free radicals in brain edema is unlikely in early reflow, because imbalance between H_2O_2-producing reactions of dopamine metabolism and mitochondrial antioxidative capacity does not occur prior to 1 hr reflow after 15 min of bilateral ischemia in gerbils. However, the findings of this study reinforce the participation of 5-hydroxytryptamine in the formation of ischemic brain edema.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02777-05 SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Cerebromicrovascular Endothelium: Distinct Peptidergic Responses *in vitro*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	M. Spatz, M.D.	Section Chief	SB, NINDS
Others:	R.M. McCarron, Ph.D.	Special Expert	SB, NINDS
	D. Stanimirovic, M.D., Ph.D.	Visiting Fellow	SB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 0.60	PROFESSIONAL: 0.30	OTHER: 0.30

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The brain capillary endothelium is the main constituent of the blood-brain barrier. This study demonstrates that various vasoconstrictive peptides evoke dissimilar responses in the endothelial cells derived from human brain (HBEC) and human umbilical vein (HUVEC). Samples of human brain surgically removed for the treatment of idiopathic epilepsy served for isolation of HBEC, whereas HUVEC were obtained commercially. Both types of cells were characterized as endothelium (>98%) by positive staining for Factor VIII-related antigen and negative staining for GFAP and other non-endothelial markers. Vasoconstrictive peptides [endothelin-1 (ET-1), arginine-vasopressin (AVP), angio-tensin II (Ang II)] markedly stimulated a transient (1-15 min) increase in IP_3 formation [EC_{50} (ET-1<AVP<Ang II)] in HBEC, while only Ang II was slightly effective on HUVEC. All three peptides also increased release of arachidonic acid (AA) from HBEC, but not from HUVEC. Both peptide-stimulated IP_3 and AA release in HBEC were inhibited by selective peptide receptor antagonists indicating the presence of ET_A, V_1, and Ang II receptors on HBEC. In contrast, HBEC reactivity to histamine was similar to that of HUVEC and consistent with the presence of histamine H1 receptors on both cell types. ET-1 (10-100 nM) increased the release of ^{51}Cr (8.2% vs. 15.7%), but not lactate dehydrogenase in HBEC only, while histamine was ineffective on either cell type. The distinct biochemical properties of EC derived from different tissues strongly suggest that these cells may be involved in the regionally diverse functions of vessels.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02797-05 SB

PERIOD COVERED

October 1, 1992 to Septemebr 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cultures of Human Cerebromicrovascular Endothelium: Mechanisms of Endothelin Effects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I	D Stanimirovic, M.D., Ph.D	Visiting Fellow	SB, NINDS
Others:	M. Spatz, M.D.,	Section Chief	SB, NINDS
	R M McCarron, Ph D	Special Expert	SB, NINDS
	A Strasser, D V M	Guest Researcher	SB, NINDS

COOPERATING UNITS (if any)

S. Uematsu, M.D., Johns Hopkins Hospital, Baltimore, Maryland

LAB/BRANCH

Stroke Branch

SECTION

Section of Neurocytobiology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.02	PROFESSIONAL: 0.55	OTHER: 0.47

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Endothelin-1 (ET-1), a member of a 21-amino acid peptide family of endothelins, can be derived from various cells of the cerebromicrovascular compartment (endothelium, smooth muscle), astrocytes, and some neurons. ET-1 is a potent and prolonged vasoconstrictor. Increased levels of ET-1 in plasma or cerebrospinal fluid of patients with hypertension, stroke, head trauma, and subarachnoid hemorrhage (SAH) have implicated ET-1 as a mediator of cerebrovascular responses in these disorders.

Recently, we described that cultured human brain endothelial cells (HBEC) secrete ET-1 in response to various vasoactive stimuli (angiotensin II, arginine-vasopressin) and express receptors for ET-1. In this study, we examined the effects of the vasoactive peptide endothelins (ET-1, ET-2, ET-3, S6b, S6c) on the release of ^{51}Cr, production of inositol triphosphate (IP_3), and release of arachidonic acid (AA) in HBEC. ET-1 induced a dose-dependent release of ^{51}Cr (EC_{50} = 7 ± 2 nM), transient increase of IP_3 (EC_{50} = 0.67 ± 0.09 nM), and sustained release of AA (EC_{50} = 59 ± 7 nM) from HBEC. Under the same experimental conditions, viability of the cells was preserved (>97%) as assessed by exclusion of the vital dye trypan blue, and release of lactate dehydrogenase.

The ET-1-induced ^{51}Cr release, formation of IP_3, and AA release from HBEC were competitively inhibited by the selective ET_A subtype receptor antagonist BQ123. ET-1-stimulated ^{51}Cr and AA release from HBEC were potentiated by protein kinase C (PKC) activator phorbol-myristate ester, and abolished by H7, an inhibitor of PKC. Dexamethasone, indomethacin, acetylsalicylic acid, imidazole, as well as the inhibitor of protein kinase A, H8, had no effect on ^{51}Cr release. The results suggest that ET_A receptor-mediated activation of PKC and increase in the HBEC 'permeability' for low molecular weight molecules in response to excessive release of endothelins from either HBEC or surrounding tissues during pathologic conditions may contribute to alterations of blood-brain barrier permeability.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

[illegible]

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Cerebromicrovascular Endothelial Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Stanimirovic, M.D., Ph.D.	Visiting Fellow	SB, NINDS
Others:	B.M. McCarron, Ph.D.	Special Expert	SB, NINDS
	M. Spatz, M.D.	Section Chief	SB, NINDS

COOPERATING UNITS (if any)

S. Uematsu, M.D., Johns Hopkins Hospital, Baltimore, Maryland; F. Bertrand, Dijon, France

LAB/BRANCH

Stroke Branch

SECTION

Section on Neurovascular Biology

INSTITUTE AND LOCATION

TOTAL STAFF YEARS: [illegible] PROFESSIONAL: [illegible] OTHER: [illegible]

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

[illegible]

[illegible]

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02860-02 SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Observations on Audiogenic Seizures in Rats Following Cardiac Arrest Cerebral Ischemia

PRINCIPAL

PI:	K. Kawai, MD	Visiting Fellow	SB/NINDS
Others:	L P Penix, M D	Visiting Scientist	SB/NINDS
	C A. Ruetzler	Biologist	SB/NINDS
	I. Klatzo, M.D.	Section Head	SB/NINDS

COOPERATING UNITS (if any)

Epilepsy Research Branch, NINDS

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.75	0.50	0.25

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our studies on ther susceptibility to audiogenic seizures (AuSz), which regularly develop 24 hr after subjecting Sprague-Dawley rats to cardiac arrest cerebral ischemia (CACI), revealed a correlation in a chronological profile between onset of seizures, changes affecting the GABAergic terminals and loss of $GABA_A$ inhibition in the hippocampus as assayed by paired-pulse stimulation (PPS) testing. The cessation of susceptibility to AuSz approximately 1 month after ischemia appears to coincide with vigorous sprouting and new formation of GABAergic terminals and return of the PPS to a normal pattern. Studies on defining sites and mechanisms of GABAergic disinhibition are associated with evaluation of how much seizures may contribute to ischemic injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 NS 02832-03SB

PERIOD COVERED
October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Immunochemical Observations on Neurotransmitter Changes in Global Cerebral Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. Kawai, M.D.	Visiting Fellow	SB, NINDS
Others:	C. Ruetzler	Biologist	SB, NINDS
	L. Nitecka, M.D.	Visiting Scientist	SB, NINDS
	J. Lohr	Biologist	SB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH
Stroke Branch

SECTION
Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFFYEARS:	PROFESSIONAL:	OTHER:
1.8	0.55	1.25

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunohistochemical observations on GABA and glutamate decarboxylase (GAD) in rats subjected to cardiac arrest cerebral ischemia (CACI) revealed strikingly early changes in the immunoreactivity of GABAergic neuronal elements expressed in the widespread swelling and increased GABA and GAD immunostaining of GABAergic terminals and boutons. These changes appeared to be generally reversible with the exception of the nucleus reticularis thalami (NRT) which showed 80% neuronal loss. GABAergic terminals in the adjacent ventral thalamic nuclei (VTN) showed, approximately 7 days after their initial disintegration, a sprouting of new terminals, which reached its peak 1 month after ischemia. This coincided with the cessation of audiogenic seizures and the return to the normal paired-pulse stimulation patterns in the hippocampus, indicating a return of $GABA_A$ inhibitory function. The hybridization assays with GAP-43 revealed strong mRNA expression limited to the NRT of rats sacrificed 7 days after CACI. The described correlations between morphologic evidence of sprouting of GABAergic terminals, and clinical cessation of susceptibility to audiogenic seizures, as well as electrophysiologic demonstration of the return of $GABA_A$ inhibitory function in the hippocampus indicate the regenerative effort of the brain tissue subjected to ischemia and provide criteria for evaluating various therapeutic measures in future studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02889-01 SB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Spreading Depression in Cardiac Arrest Cerebral Ischemia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	N. Kawahara, M.D.	Visiting Fellow	SB, NINDS
Others:	L.P. Penix, M.D.	Visiting Fellow	ERB, NINDS
	C.A. Ruetzler	Biologist	SB, NINDS
	I. Klatzo, M.D.	Section Chief	SB, NINDS

COOPERATING UNITS (if any)

Epilepsy Research Branch, NINDS

LAB/BRANCH

Stroke Branch

SECTION

Cerebrovascular Pathophysiology Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	1.75	PROFESSIONAL:	0.50	OTHER:	0.25

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The role of the spreading depression (SD) was investigated in rats subjected to cardiac arrest cerebral ischemia (CACI). The SD was induced by application of KCl either on the exposed dura of the parietal cerebral cortex or by KCl perfusion through the hippocampus. Three days later, the animals underwent the CACI. With regard to the hippocampus unilateral perfusion with KCl regularly resulted in induction of the SD in the ipsilateral hippocampus associated with marked elevation of glutamate. No such effect was observed in rats in which KCl had been substituted with physiologic saline solution. Animals with hippocampal KCl perfusion, followed 3 days later by CACI, showed significant protection of CA1 pyramidal neurons on the side of the perfusion. No such effect was observed in Krebs-Ringer perfused rats. The protective effect of KCl on CA1 pyramidal neurons was evident also following the cortical application, although the effect was more bilateral. The SD induced 3 days before CACI resulted in a marked reduction in the susceptibility of rats to audiogenic seizures (AuSz) when tested 24 hr after cardiac arrest insult.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02821-04SB

PERIOD COVERED

October 1, 1992 to September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Dynamics of Postischemic Calcium Accumulation and Protein Synthesis in Brain Tissue

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G Mies, M.D.	Visiting Associate	SB/NINDS
Others:	K Kawai, M.D	Visiting Fellow	SB/NINDS
	I Klatzo, M D	Chief	SB/NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Stroke Branch

SECTION

Section of Cerebrovascular Pathology

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	0 50	PROFESSIONAL:	0 25	OTHER:	0.25

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Dynamics of pathological changes in brain tissue following cardiac arrest induced ischemia related to the findings of double tracer autoradiography of ^{45}Ca and [^{3}H] leucine uptake as respective indicators of ischemic injury and metabolic disturbance. Abnormal calcium accumulation, determined by ^{45}Ca uptake, was related to injured but still living neurons and to reactive glial elements. ^{45}Ca autoradiography confirmed a high sensitivity to neuronal injury of the nucleus reticularis thalami (NRT), hippocampal CA1 pyramidal layer, inferior colliculus, ventral thalamic nucleus (VTN), caudate nucleus and parietal cortex. [^{3}H]leucine incorporation revealed that an initially widespread inhibition of protein synthesis was followed by its considerable recovery. Observations concerning the hippocampal CA1 sector and VTN suggested that a significant degree of protein synthesis, maintained at the late stage after postischemic recovery, was related to survival and regeneration of neurons and not to the presence of glial elements.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02454-13 SN

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Human Pituitary Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Edward Oldfield, M D Chief, SNB, NINDS

COOPERATING UNITS *(if any)*

Developmental Endocrinology Branch, NINDS
Diagnostic Radiology, CC

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, CNP

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS: 1 0	PROFESSIONAL: 1 0	OTHER: 0 0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects [x] (b) Human tissues [ ] (c) Neither

[ ] (a1) Minors

[ ] (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We investigated venous sampling of the pituitary venous drainage to aid in the diagnosis and treatment of patients with Cushing's syndrome. Over 700 patients have now received bilateral simultaneous inferior petrosal sinus (IPS) sampling. The results indicate that 1) the procedure can be performed successfully in all patients with Cushing's syndrome (successful sampling has been performed in over 99% of the patients in whom it has been attempted); 2) the procedure distinguishes patients with ectopic ACTH secretion from those with pituitary adenomas with nearly 100% accuracy; 3) IPS sampling successfully determines in which side of the pituitary gland microadenomas reside in patients with Cushing's disease with 70% accuracy, and 4) unilateral inferior petrosal sinus sampling, which is commonly used clinically, is frequently misleading.

Repeat transsphenoidal surgery is successful in eliminating the hypercortisolism of Cushing's disease in about 70% of patients. This therapy for patients with Cushing's disease after previous pituitary surgery had not previously been examined. Repeated sella exploration in the early postoperative period in patients who did not respond to the first operation was shown to be successful in most patients who received it. The subset of patients who are most likely to have success with early repeat surgery can be selected based on the findings during the first operation.

MRI scanning with and without gadolinium-EDTA was used to evaluate patients with Cushing's disease preoperatively. This technique permitted identification of the adenoma in about 55% of those patients with surgically proven microadenomas. Proper timing of the MRI after administration of gadolinium-EDTA was critical in the optimal use of the technique. Pituitary adenomas were detected in 10% of 100 normal subjects with MRI scanning with contrast.

The endocrine aberration in pituitary tumors and ectopic ACTH secreting tumors that cause Cushing's syndrome is loss of normal negative feedback regulation by cortisol. To investigate the basis of this, the structure of the pro-opiomelanocortin promoter region was investigated in pituitary and extrapituitary ACTH- producing tumors and demonstrated to be normal.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02674-09 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Monoclonal Antibody-Toxin Conjugates for Tumor Therapy *in vivo*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Youle, Ph.D	Chief, Biochemistry Section, SNB, NINDS
Other:	
Dianne Newton, Ph.D	Staff Fellow, SNB, NINDS
Susanna Rybak, Ph.D	Special Expert, SNB, NINDS
Massimo Gadina, Ph.D	Special Volunteer, SNB, NINDS
You-Neng Wu, Ph.D	Visiting Associate, SNB, NINDS

COOPERATING UNITS (if any)

Alfacell, Bloomfield, New Jersey

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
4.0	4.0	0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Monoclonal antibodies selectively bind tumor cell differentiation antigens *in vitro* and *in vivo*. Since natural effector mechanisms often do not mediate killing of monoclonal antibody bound cells we have devised methods of linking extremely toxic proteins to the antibodies to selectively kill tumor cells.

We have succeeded in developing several new approaches to apply immunotoxins *in vivo*. 1) Cloning toxins, then altering their structure at the gene level to decrease non target cell toxicity; 2) intrathecal administration of immunotoxins for therapy of brain tumors that kill 2-5 logs of tumor cells in animal models; 3) preparation of genetically engineered immunotoxins for clinical trials of human brain tumor patients; 4) prevention of an immune response against immunotoxin with anti-CD4 antibodies, 5) specific deletion of Purkinje cells in rats, guinea pigs, and rhesus monkeys; 6) use of human cytotoxic proteins such as RNase linked to antibodies to selectively target cells; and 7) understanding the mechanism of human RNase neurotoxins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02697-09 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protection of the Brain Against Injury by Ionizing Radiation with Pentobarbital

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	
Aytac Akbasak	Visiting Associate, SNB, NINDS
Calvin Hawkins	Bio Lab Technician, SNB, NINDS

COOPERATING UNITS (if any)

National Cancer Institute, Radiation Oncology Branch

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was completed 10/92

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02708-08 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Vascular Permeability Factor/Vascular Endothelial Growth Factor in the CNS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Marsha Merrill, Ph D., Biologist, SNB, NINDS

Others:

John Heiss, M.D., Senior Staff Fellow, SNB, NINDS,
Nancy Edwards, B.A., Biologist, SNB, NIND
Efstathios Papavassiliou, M.D., Visiting Fellow, NINDS
Edward H. Oldfield, M.D., Chief, SNB, NINDS
Mirna Bacic, M.D., Visiting Associate, SNB, NINDS
Seth Zeidman, M.D., Clinical Associate, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Tumor Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF), has been proposed to be a mediator of endothelial proliferation and angiogenesis in normal and diseased states, and to have a role in the development of tumor-associated vascular hyperpermeability. The purpose of this study was to examine expression of the VPF/VEGF gene in both tumor and normal tissues.

In a study of the levels of VPF/VEGF mRNA in 42 CNS neoplasms and 7 normal human brain samples, significantly higher levels (up to 10-fold higher) were observed in those tumors commonly associated with vascularity or cerebral edema (glioblastoma multiforme, hemangioblastoma, meningiomas). In those tumors not associated with increased vascularity and edema (pituitary adenomas and nonastrocytic gliomas), the levels of VPF/VEGF were not significantly different from those in normal brain. Cloning and sequencing of PCR-amplified GBM and normal brain cDNA demonstrated three forms of the VPF/VEGF coding region corresponding to mature polypeptides of 189, 165, and 121 amino acids, respectively. The relative abundance of the forms of VPF/VEGF mRNA was consistent in tumor and normal brain. Absorption of capillary permeability activity from human glioblastoma multiforme (GBM) cell conditioned medium and GBM cyst fluids by anti-VEGF antibodies demonstrated that VEGF is secreted by GBM cells and is present in sufficient quantities *in vivo* to induce vascular permeability.

We used Northern blot analysis and *in situ* hybridization histochemistry to establish that VPF/VEGF mRNA is expressed in the brain, kidney, liver, lung, and spleen of the adult rat. On Northern blots, the relative abundance of VPF/VEGF mRNA observed in these tissues was highest in the lung and lowest in the spleen. As determined by *in situ* hybridization, the patterns of VPF/VEGF expression are organ-specific. Cloning studies in the rat demonstrate that multiple forms of VPF/VEGF are also expressed in the rat.

The widespread expression and organ-specific distribution of VPF/VEGF mRNA in normal rat tissues, and the increased expression in human central nervous system tumors, suggest an extensive role for this factor in the physiology of both normal and tumor vasculature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02739-07 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Laboratory Investigation of Central Nervous System Vascular Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D., Chief, SNB, NINDS

Others:

Ryszard Pluta, M.D., Visiting Associate, SNB, NINDS
Robert Boock, Ph.D., Staff Fellow, SNB, NINDS
Marston Linehan, M.D., Surgical Branch, NCI
Berton Zbar, M.D., Senior Investigator, NCI
Tom Manski, M.D., National Naval Medical Ctr
Kourosh B. Afshar, M.D., CA, SNB, NINDS

COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC, Experimental Therapeutics Branch, NINDS
Surgery Branch, National Cancer Institute; National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL 4.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Endothelial-derived relaxation factor nitric oxide (NO) was shown to mediate autoregulation and chemoregulation of cerebral blood flow. NO synethase immunoreactivity was demonstrated in the nerve plexus in the adventitia of the circle of Willis in primates. In a primate model of subarachnoid hemorrhage (SAH) adventitial disappeared on day 7 with the development vasospasm and did not return on day 14 with resolution of vasospasm, suggesting that NO loss plays a central role in the pathogenesis of cerebral vasospasm after SAH. Thus, direct replacement of NO should reverse the vasospastic effect of any NO loss. In the primate model of vasospasm, intra-arterial infusions of NO solution reversed arteriographic cerebral vasospasm, significantly increased cerebral blood flow, and decreased cerebral blood flow velocity. These findings further support a cental role of NO in the pathogenesis of cerebral vasospasm and suggest the potential of a regional NO therapy for cerebral vasospasm.

We have explored the effects of the putative agents of vasospasm, oxyhemoglobin and its breakdown product methemoglobin in cell culture. These cultures studies are used to examine the possibility that vasospastic agents (e.g., endothelin) may be released from tissues exposed to oxyhemoglobin and methemoglobin. The first studies in this series have shown that astrocytes die when exposed to oxyhemoglobin in culture. This suggests that (1) oxyhemoglobin-induced endothelin release is unlikely to underlay cerebral vasospasm, and (2) cerebral injury and the production of seizures after intracerebral hemorrhage may result from distinct glial toxicity induced by oxyhemoglobin.

A specific type of cranial dural arteriovenous fistulas was identified and shown to be treated effectively by simple interruption of the intrathecal venous drainage, a much simpler and safer procedure than the surgical procedure previously used to treat these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02781-06 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Tissue Implantation in Parkinsonian Models

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	
Daniel Lieberman, M.D.	Staff Fellow, SNB, NINDS
Alex Cummins, M.S.	Biologist, SNB
Hideki Takubo, M.D.	Visiting Associate, NINDS

COOPERATING UNITS (if any)

David Jacobawitz, Clinical Neuropharmacology, NIMH, Charles Gerfen, Neurophysiology, NIMH, Ivan Mefford, Neurochemistry, NIMH

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

CNS Transplantation Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 6.5	PROFESSIONAL: 6.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Brain grafting has emerged as a novel therapy for patients suffering from Parkinson's disease who are refractory to medical therapy. Behavioral recovery following caudate cavitation in Parkinsonian monkeys has focused our attention on potentially beneficial host responses to grafting. Following injury the CNS produces neurotrophic factors which promote neurite outgrowth and glial proliferation. We have explored the therapeutic potential of growth factors in preventing or reversing biochemical and behavioral parameters in rodent models of Parkinson's disease. To further investigate the physiology of nerve growth factors we have lesioned the dopamine system in mice with MPTP and measured the transcription of brain-derived neurotrophic factor (DBNF) and neurotrophin-3 using Northern blotting.

To deliver growth factors to gray matter we are developing methods for convection-enhanced direct infusion and ex vivo gene therapy. We used convection to enhance the distribution of large molecules injected into the striatum in rats, measured using immunohistochemistry and quantitative autoradiography. We are beginning to explore the viability and biology of fetal human glial cells after transplant in mice, rats, and monkeys as an alternative paradigm to continuously deliver proteins to the degenerating dopamine system.

Recent electrophysiologic and anatomic studies have shown hyperactivity of neurons in the subthalamic and globus palladium interna nuclei produce the symptoms of Parkinson's disease. Accordingly, we are exploring the use of excitatory amino acids to destroy the globus pallidus interna in monkeys as a novel therapy.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02812-04 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pentobarbital Effects on Damage of the Primate Brain by Fractionated Whole Brain Radiation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D	Chief, SNB, NINDS
Others:	
Aytac Akbasak, M.D.	Visiting Associate, SNB, NINDS
Tom Goffman, M.D.	Radiation Oncology Branch, NCI
Kathryn Orr, R.N.	Radiation Oncology Branch, NCI
Calvin Hawkins	Bio Lab Technician, SNB, NINDS
Lisa Berney	Office of the Director, NINDS

COOPERATING UNITS (if any)

Radiation Oncology Branch, NCI

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER 0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Radiation therapy remains the single most effective treatment for malignant brain tumors, but in many cases, toxicity to normal brain impedes therapeutic doses sufficient for local control to be achieved. A substantial effort has been directed toward overcoming the unfavorable side effects of brain tumor radiation therapy.

Data from our institute and others indicate that the concomitant application of pentobarbital anesthesia during cerebral irradiation reduces the toxicity of the ionizing radiation. Although mechanisms of this phenomenon remain unclear, it seems to arise from general suppression of brain synaptic activity or metabolism.

After baseline MRI scans of the brain and neuroendocrine testings, primates (*Macaca mulatta*) undergo whole brain X-irradiation in 10 daily fractions, 360 rads each, total dose of 3600 rads. The monkeys in the study group were anesthetized with pentobarbital during the irradiation whereas the animals in the control group received ketamine. Each group consists of 6 animals. Neuroendocrine testing and MRI scan follow-up studies are performed at 3, 6, 12, 18 and 24 months after irradiation. Quantitative histology will be done on the capillary bed, glial and neuronal populations after sacrifice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02813-04 SNB

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacokinetics of Direct Brain Infusion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D	Chief, SNB, NINDS
Others:	
Douglas W. Laske, M.D	Senior Staff Fellow, SNB, NINDS
Orhan Ilercil, M.D.	Clinical Associate, SNB, NINDS
Aytac Akbasak, M.D	Visiting Associate, NINDS
Bob Boock, Ph.D.	Senior Staff Fellow, SNB, NINDS
Paul Morrison, Ph.D., Robert Dedrick, Ph.D	Biomedical Engineering, RR

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [ ] (b) Human tissues
- [x] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For many compounds (neurotrophic factors, antibodies, growth factors, genetic vectors, enzymes) minimal diffusion in the brain severely limits drug distribution after direct drug administration in to brain parenchyma. We systemically investigated convection, molecular transport with bulk flow of fluid, to enhance the distribution of large and small molecules, indium^{111}-transferrin (In^{111}-Tf; MW 80,000) and C^{14}-sucrose (MW 359), by maintaining a pressure gradient during interstitial infusion to generate bulk flow through the brain interstitium. The volume of distribution (V_d) containing $\geq$ 1% of infusate concentration increased linearly with the infusion volume (V_i) for In^{111}-Tf ($V_d/V_i = 6.1$) and C^{14}-sucrose ($V_d/V_i = 14.1$). 24 hr after infusion, the distribution of In^{111}-Tf increased, became more homogeneous, and penetration into gray matter occurred. By using convection to supplement simple diffusion, greatly enhanced distribution of large and small molecules can be achieved in the brain while achieving drug exposure orders of magnitude greater than systemic exposure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 NS 02814-04 SNB

PERIOD COVERED
October 1, 1992 Through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic Abnormalities in Primary Glial Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Iqbal U. Ali, Ph.D. SNB, NINDS

Others:
Abha Saxena, Ph.D., Visiting Associate, SNB, NINDS
Barbara Ikejiri, B.S., Biologist, SNB, NINDS
Cindy Piccirilli, M.D, Resident, NNMC
Edward H Oldfield, M.D., Chief, SNB, NINDS
William Stettler-Stevensen, M D, NCI
James Robertson, M.D., Chairman, Dept of NS, University of Tennessee

COOPERATING UNITS *(if any)*
University of Tennessee, Memphis, Tennessee
LCMB, NCI and the NNMC, Bethesda, Maryland

LAB/BRANCH
Surgical Neurology Branch, NINDS

SECTION
Molecular Biology Unit, SNB, NINDS

INSTITUTE AND LOCATION
NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0

CHECK APPROPRIATE BOX(ES)
[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Glioblastomas are extremely complex and malignant neoplasms. We have taken several approaches to understand and identify, at a molecular level, the underlying mechanisms that translate into the malignant behavior of these tumors.

1. Loss of heterozygosity of several markers on chromosomes 17 and 10 was detected in a significant number of glioblastomas. The p53 gene was deleted and/or mutated in 75% of the tumors with gene losses on chromosome 17. Deletion mapping studies on chromosome 17 suggested the presence of another potential tumor suppressor gene distinct from the p53 gene.
2. Immunohistochemical analysis of the p53 protein showed a heterogeneous pattern of subcellular compartmentalization in glioblastomas. Tumors with one single wild type allele of p53 and tumors with one wild type and one mutant allele of p53 and gene losses on chromosome 17p distal to p53 show increased cytoplasmic and/or nuclear accumulation of p53. Furthermore, tumors with mutations in the same codon of p53 display very different staining patterns. These data suggest that the microenvironment of a particular tumor is important in determining the subcellular localization of p53.
3. Twenty tumors were analyzed for collagenase IV and Timp-2 expression. Both these genes were generally over-expressed in glial tumors compared to the normal human brain.
4. Six matched pairs of primary and recurrent tumors were analyzed for allelic deletions on chromosomes 10 and 17 and other genetic alterations. The data clearly demonstrated additional genetic abnormalities in recurrent tumors, which included amplification of the α PDGRF gene, point mutations of the p53 gene and overexpression of collagenase.
5. Analysis of metastatic brain tumors showed chromosome 17p deletions and/or p53 mutations in 60% of the tumors. Our data support the concept that p53 gene alterations may contribute to the metastatic spread in certain types of cancers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02815-04 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Pituitary Conticotroph Adenomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI: Iqbal Ali, Ph.D.	SNB, NINDS
Others:	
Joan Barrick, B S	Biologist, SNB, NINDS
Barbara Ikejiri, B S	Biologist, SNB, NINDS
Edward Oldfield, M D	Chief, SNB, NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cushing's disease is caused by the pituitary hypersecretion of ACTH and occurs predominately in women Patients are cured by surgical removal of an ACTH-producing adenoma, suggesting evolution and expansion of a genetically aberrant cell However, hypothalamic dysfunction and excessive stimulation of anterior pituitary corticotrophs by one or more neurotransmitter substances may also lead to the development of corticotropic adenomas

Allelotyping of the pituitary tumors is being carried out by using restriction fragment length polymorphism (RFLP) analysis Initial studies showed loss of heterozygosity of genes on chromosome 17 in one of the 4 Nelson's tumors

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02B23-04 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antibody-Toxin Conjugates for the Treatment of Human Brain Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Youle, Ph.D	Chief, Biochemistry Section, SNB, NINDS
Doug Laske, M.D.	Senior Staff Fellow, SNB, NINDS
Orhan Ilercil, M.D.	Clinical Associate, SNB, NINDS
Edward H. Oldfield, M.D.	Chief, SNB, NINDS
David Katz, M.D	Neuropathologist, OD, NINDS
Cynthia Sung, Ph.D.	Staff Fellow, PEIB
Robert Dedrick, Ph.D	Senior Staff Fellow, PEIB

COOPERATING UNITS (if any)

Diagnostic Radiology, Nuclear Medicine Department, National Cancer Institute, Hafslund Nycomed

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 2.5	PROFESSIONAL 2.5	OTHER 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[ ] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A phase I dose-escalation study of intrathecal therapy with the immunotoxin 454A12-RTA for leptomeningeal neoplasia has been completed. This compound is a conjugate of a monoclonal antibody against the human transferrin receptor and the recombinant ricin A chain protein toxin. Eight patients with leptomeningeal spread of systemic neoplasia were treated with a total of 10 different doses of intrathecal immunotoxin covering a 1000-fold increase in drug dose (1.2 to 1200 micrograms).

No toxicity was detected until the highest doses were reached. Acute toxicity consisted of transient headache, vomiting and decreased mental status with elevated intracranial pressure which was responsive to steroids and cerebrospinal fluid (CSF) drainage. Bioassays of serial CSF samples from these patients against tumor cell lines *in vitro* revealed that patients CSF retained cytotoxic activity against tumor cells for approximately 48 hours after intraventricular administration of immunotoxin. In addition, *in vitro* testing of 454A12-RTA against tumor cells harvested from the CSF in 3 study patients revealed tumor cell sensitivity to the drug before and after treatment at concentrations of drug much lower than the concentration achieved in CSF. Four patients had decreased lumbar CSF tumor cell counts, the most dramatic (>95%) occurring at the highest dose given.

These results indicate that immunotoxin can be safely administered intrathecally in humans, retain bioactivity in the CSF, are cytotoxic to tumor cells from patients, and can reduce tumor burden after only a single dose.

A new clinical trial of a genetically engineered immunotoxin, Tfn-CRM107, discovered with the branch has begun for treatment of parenchymal brain tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02840-03 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Analysis of Alpha Subunits of G Proteins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Iqbal Ali, Ph.D SNB, NINDS

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Molecular Biology Unit

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Components of signaling pathways that promote proliferation are likely to play a central role in normal cellular growth and differentiation, and are therefore potential targets during pathogenesis, especially neoplastic growth. Guanine nucleotide binding (G) proteins are membrane-associated heterotrimers (alpha, beta, and gamma subunits) and play an important role in transmembrane signal transduction. One type of alpha subunit, Gs, is ADP-ribosylated by cholera toxin and mediates activation of adenylate cyclase. Two point mutations, found at the cholera toxin ribosylation site and a proposed conformational switching area (S-box), have been proposed to be oncogenic in a subset of growth hormone-producing pituitary adenomas.

We have carried out G-specific PCR amplification and subsequent cloning of amplified cDNAs from normal human brain tissue, placenta, an SV40-transformed human astroglial cell line, a glioblastoma cell line, (HS683) a primary glioblastoma, and an ACTH-producing pituitary adenoma. Characterization of the recombinant clones showed the presence of novel truncated Gs transcripts in the transformed astroglial cell line SVG, glioblastoma cell line HS683, and glial and corticotroph tumors. Our results suggest that aberrant splicing of Gs may have a modulatory function in transformation.

This project was completed January 1993

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02850 - 02 SNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Gene Therapy for Brain Tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Others:	
Zvi Ram, M.D	Visiting Scientist, SNB, NINDS
Stuart Walbridge	Biologist, SNB, NINDS
Kenneth Culver, M D	Senior Clinical Investigator, MB, NCI
R. Michael Blaese, M D	Chief, Cellular Immunology, MB, NCI

COOPERATING UNITS (if any)

National Cancer Institute, Bethesda, Maryland
Genetic Therapy, Gaithersburg, Maryland

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigate the application of gene therapy for the treatment of malignancies of the CNS. Several different projects constitute that goal and include: a clinical trial using *in vivo*, retroviral-mediated, transfer of the herpes simplex thymidine kinase gene (HStk) into malignant brain tumors followed by intravenous ganciclovir, preclinical study of HStk-gene transfer for the treatment of meningeal carcinomatosis, preclinical studies of other viral vectors for gene therapy, and studies of the distribution mechanics of viral-sized particles in normal brain and tumors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02854 - 02 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Establishing the Physiology of Syringomyelia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Edward H. Oldfield, M.D. Chief, SNB, NINDS

Others:

John D. Heiss, M.D., Senior Staff Fellow, SNB
Charles Haworth, M.D., LCDR, MC, USNR
Thomas Shawker, M.D., CC Radiology
William Kammerer, M.D., CC Anesthesiology
Thomas Talbot, RR, BEIP
Morris Pulliam, M.D., Capt, MC, USN
Nick Patronas, M.D., CC, Radiology
Robert Dedrick, Ph.D., RR, BEIP
Alec Eidsath, Ph.D., RR, BEIP

COOPERATING UNITS (if any)

Diagnostic Radiology Department, CC
Anesthesiology Department, CC, BEIP

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
[ ] (a1) Minors
[ ] (a2) Interviews
[x] (b) Human tissues
[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to establish the mechanism(s) of progression of communicating syringomyelia. Communicating syringomyelia usually accompanies abnormalities at the craniocervical junction. Measurement of intraventricular pressure, intrathecal pressure, and intrasyrinx pressure provide data which elucidate the hydrodynamic mechanism(s) of progression of syringomyelia. Radiographic testing, including MRI flow studies, ultrasonography, and Imatron CT, is demonstrating how pathologic anatomy alters normal CSF. The effect of posterior fossa craniectomy, upper cervical laminectomy, and duraplasty on CSF flow, syrinx size, and neurologic function is being evaluated.

Five patients have been treated. No patient had communication between the 4th ventricle and the syrinx. Ultrasonographic measurements demonstrated cord and syrinx constriction during systole. Despite obstruction of CSF pathways at the foramen magnum, phase and cine-MRI demonstrated pulsatile syrinx and cervical subarachnoid CSF flow. CSF pressure measurements confirmed the transmission of intracranial pressure to the cervical subarachnoid space and the syrinx. Because intracranial pressure is transmitted despite obstruction of the subarachnoid space at the foramen magnum, we conclude that the cerebellar tonsils and the brainstem act on a partially enclosed spinal subarachnoid space to generating cervical subarachnoid CSF pressure waves. These waves compress the spinal cord from without, not from within, as has previously been considered to occur, to propel the syrinx fluid downward with each heart beat. Syrinx progression occurs as a consequence.

Craniocervical decompression and duraplasty improved CSF at the foramen magnum in all patients. The syringes decreased in size following surgery. The pressure measurements have been performed without complication. We plan to proceed with an additional 5 patients.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02855-02 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interstitial Therapy with Targeted Protein Toxins for Malignant Brain Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Douglas Laske, M.D.	Senior Staff Fellow, SNB, NINDS
Others:	
Eward H. Oldfield, M.D.	Chief, SNB, NINDS
Richard J. Youle, Ph.D.	Chief, Biochemistry Section, SNB, NINDS
Orhan Ilercil, M.D.	Clinical Associate, SNB, NINDS
David Katz, M.D.	Neuropathologist, OD, NINDS
Nicholas Patrons, M.D.	Radiologist, CC

COOPERATING UNITS *(if any)*

Department of Radiology, CC

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[ ] (b) Human tissues

[x] (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are investigating a new experimental approach for the treatment of malignant brain tumors which utilizes a new class of potent, targeted anticancer compounds, called immunotoxins. We have initiated a dose escalation trial of regional therapy with the immunotoxin transferrin-CRM107 (Tf-CRM107) for the treatment of recurrent malignant brain tumors. Tf-CRM107 is a conjugate of human transferrin (Tf) and and diphtheria toxin with a point mutation (CRM107). Tf-CRM107 binds to the transferrin receptor, which facilitates iron uptake and is present in higher number on tumor cells than on the normal cells of the brain, and the diphtheria toxin mutant kills these tumor cells to which the Tf-CRM107 binds. The purpose of the study is to evaluate the toxicity of Tf-CRM107 when delivered by intra- and peritumoral slow interstitial infusion in a dosage-escalation schedule, and to assess antitumor activity in these patients.

Ten patients with malignant brain tumors (5 glioblastoma, 3 anaplastic astrocytoma, 2 metastatic lung carcinoma) that have failed standard therapy (surgical resection or biopsy, radiation therapy, and chemotherapy in some), with evidence of tumor progression, have been treated. For treatment, single or multiple silastic infusion catheters were stereotactically placed intratumorally and Tf-CRM107 was infused over 2-6 days using an external syringe pump (rates 0.5-6.0 µl/min). The initial Tf-CRM107 concentration was 7×10^{-10}M which has been increased by 1/2 log increments every 4 patients; the last patient was treated with 7×10^{-9}M Tf-CRM107. Total dose has increased from 0.05 to 27.3 µg. Patients were to be treated monthly, and of 19 total treatments, 5 patients have been treated twice and 1 patient has been treated 5 times.

Tf-CRM107 infusions were well tolerated with no severe drug-related neurologic or systemic toxicity identified to date. Three patients suffered transient worsening of a neurologic--deficit that resolved with steroids and/or mannitol. Two seizures (1 generalized, 1 focal) occurred during a total of 19 treatments. Two patients required increased steroid dosages after treatment due to prolonged increased peritumoral edema. The only systemic effect of treatment detected has been a mild transient elevation of the liver enzyme SGPT in 6 of 10 patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02859-02SN

PERIOD COVERED

October 1, 1992 - September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Programmed Cell Death in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Richard J. Youle, Ph.D. — Chief, Biochemistry Section, SNB, NINDS

Bruno Dipasquale, M.D. — Visiting Associate

Katherine A. Wood, Ph.D. — Visiting Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Biochemistry Section

INSTITUTE AND LOCATION

NINDS, National Institutes of Health, NINDS

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
3	3.0	0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have begun to study programmed cell death in the nervous system and the biochemical mechanism of apoptosis in general. To approach the nervous system more sensitive and in situ methods are needed to identify cellsundergoing programmed cell death. We have developed two new methods to identify apoptotic cells under the microscope. 1) We have found that thymocyte programmed cell death can be followed morphologically with Nomarski optics and that the thymocyte death resembles neuronal cell death. The morphologic analysis of nuclear disintegration has allowed us to test whether cell death is due to production of a toxic factor or due to the loss of a protective factor. Using the new microscopic method to identify apoptosis, the nuclei in the heterokaryons were found to follow the original and distinct fate of the parent cells and not to transfer apoptosis nor viability between nuclei. This new method also allowed us to identify apoptosis as the method of cerebellar granule cell death after MPP+ treatment in vitro. 2) We have also developed a molecular detection method to measure DNA strand breaks in situ. This allows us to examine brains of animals undergoing neurodegenerative changes during ischemia, MPTP treatment, and during development. This new method should illuminate the role apoptosis plays during development and during various disease states of the nervous system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02868-02 SNB

PERIOD COVERED

October 1, 1992 Through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Semi-Chronic Intracortical Electrical Stimulation of the Visual Cortex of a Blind Volunteer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Conrad Kufta, M.D	Medical Officer, SNB, NINDS
Others:	
Daniel O'Rourke, M.D.	Clinical Associate, SNB, NINDS
Martin Back	Electrical Engineer, LNLC, NINDS
Edward Schmidt, Ph.D	Biological Engineer, LNLC, NINDS
F. Terry Hambrecht, M.D.	Head, Neuroprothesis, NINDS

COOPERATING UNITS (if any)

Howard Hughes Fellow - P. Vallabhanath

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

[x] (a) Human subjects
- [ ] (a1) Minors
- [ ] (a2) Interviews

[x] (b) Human tissues

[ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is designed to evaluate the feasibility of a visual prosthesis for totally blind individuals by stimulating chronically implanted microelectrodes in the visual cortex. A 42-year-old woman who has been blind for 22 years was implanted with an array of 38 electrodes in the visual cortex. Stimulation of individual electrodes produced sensation of light called phosphenes. Phosphenes were produced with 34 of the 38 electrodes with currents that were 100 to 1000 times lower than had been reported for surface stimulation of the visual cortex. Additional blind patients need to be tested before we will know if intracortical microstimulation (ICMS) of the visual cortex is a feasible technique for producing a visual prosthesis. However, all the tests performed to date indicate that ICMS may be feasible.

DEPARTMENT OF HEALTH AND HUMAN S... /ICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02880-01 SNB

PERIOD COVERED

October 1, 1992 through September 30, 1993

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Inducers of differentiation of malignant brain tumors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Zvi Ram, M.D.	Visiting Scientist, SNB, NINDS
Others:	
Edward H. Oldfield, M.D.	Chief, SNB, NINDS
Stuart Walbridge, B.S.	Biologist, SNB, NINDS
John Viola, M.D., Eric Oshiro, M.D.	Clinical Associate, SNB, NINDS
Dvorit Samid, M.D.	Clinical Pharmacology Branch, NCI
Charles Myers, M.D.	Clinical Pharmacoogy Branch, NCI

COOPERATING UNITS (if any)

National Cancer Institute

LAB/BRANCH

Surgical Neurology Branch, NINDS

SECTION

Clinical Neurosurgery Section, SNB, NINDS

INSTITUTE AND LOCATION

NINDS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0.0

CHECK APPROPRIATE BOX(ES)

- [ ] (a) Human subjects
  - [ ] (a1) Minors
  - [ ] (a2) Interviews
- [x] (b) Human tissues
- [ ] (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We investigated the *in vitro* and *in vivo* effect of phenylacetate (NaPA) on experimental malignant brain tumors. Phenylacetate is a nontoxic natural compound that induces maturation of malignant glial cells by depleting plasma glutamine and blocking metabolic pathways necessary for malignant growth. We showed that NaPA inhibits proliferation of tumor cells (*in vitro* and *in vivo*), is associated with profound cell maturation, and extends survival when given as a preventive or therapeutic treatment.